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STRATEGIES FOR IMPROVING SOIL FERTILITY IN ALFALFA STANDS

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Strategies for Improving Soil Fertility in Alfalfa Stands

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a) Abstract/Summary

This study examined the feasibility of applying microbial inoculants (for N fixation and P solubilization) and N, P and S fertilizers to post-emergent crops. In the first study, fertilizers and inoculants were applied to aging alfalfa stands (5 years old or older) in an attempt to improve productivity of the stand. The inoculants and P fertilizers were placed in the soil using a small plot disc seeder in the early spring. Three sites in the northern alfalfa producing area of the province were established. The seeder itself did not cause significant damage to the stands. At two of the sites (Smeaton and Star City), simply disturbing the soil by running the seeder over the plots improved productivity slightly. Soil bulk density measurements indicated that both of these sites were relatively compacted. The third site at Crooked River did not respond to the disturbance. This site was the least compacted of the three sites. The Star City site was the least fertile of the sites and responded to more of the treatments than the other two sites. Star City was severely S deficient and responded strongly to the ammonium sulphate fertilizer, as well as the small amount of S (7 kg ha^{-1}) applied with the gypsum in the inoculant carrier. All of the sites showed a tendency to increase productivity associated with the application of S. Although knowledge that alfalfa is a heavy user of S is not new, this study does indicate that in the northern region of the province where all of the sites were located, that S deficiency might be more widespread than is recognized. Star City was the only site to respond positively to reinoculation with rhizobia. Although soil tests for P and N were low to marginal, there was no significant, widespread response to either of these fertilizers. Inoculation with *P. bilaiae* in some cases actually decreased yield. Mature alfalfa stands in general continue to acquire enough N to sustain the crop, and are efficient at extracting soil P to meet their needs.

In the second study, rhizobia and *P. bilaiae* were applied either in the year of seeding a new alfalfa stand, or delayed by one year, simulating an inoculant failure, or failure of the producer to inoculate. Overall, delaying the application of the inoculants did not affect productivity, nor N and P fertility in the crop.

Introduction

Forage production in Saskatchewan is increasing and is expected to continue increasing as farmers look for ways of diversifying and reducing production and transportation costs. In the 2000-2001 crop year, acreages of land seeded to tame hay were 15% higher than the 5 year average, and 43% higher than the 20 year average (SAF, 2000). Initiatives that will contribute to the expansion of the forage industry include the goal of increasing beef cow herds to a projected 3.3 million head by the year 2010, up from 2.2 million head in 2000 (SAC, 2000). Clearly, land usage will need to be optimized in order to feed these higher herd numbers. In addition, the Saskatchewan government has implemented permanent cover initiatives to convert marginal farmlands back to more permanent pasture conditions. The permanent cover incentive is aimed at resource conservation as well as soil sequestration of carbon as one strategy to reduce agriculture's contribution to greenhouse gas emissions.

Among the crops seeded for tame hay production, several are forage legumes, of which alfalfa, sweet and red clovers are the most common. Despite the availability of other "bloat-free" forages, alfalfa continues to be the most commonly grown pasture legume in both Alberta and Saskatchewan (Alberta Agriculture, Food and Rural Development, 1996). Its popularity is due to its nitrogen fixing ability, high yield and high nutrient value as well as its adaptation to a wide range of climatic and soil conditions including moderate drought tolerance and winter hardiness. It also performs reasonably well under irrigation. Alfalfa and other forage legumes also are major rotational crops in organic management systems, capitalizing on their nitrogen fixing capabilities. Simply growing alfalfa can reduce chemical labor and equipment inputs, potentially saving the producer money. For marginal farmlands where annual crop production is neither economically nor environmentally sustainable, conversion of the land to forage production is an attractive alternative

Alfalfa can be grown for hay or pasture, seed, or "dehy" production. Alfalfa for hay is typically seeded as a mixed forage stand with a grass, most commonly smooth brome. Seed and dehy are grown as pure stands. Typically, alfalfa fields produce maximum yields for the first few years after establishment, until fertility and moisture levels decrease. Seed alfalfa is much less nutrient demanding than alfalfa grown for hay,

and stands tend to remain vigorous for longer periods of time. Although it is a recommended practice, many producers (particularly those of the older generation) do not inoculate the seed with a commercial *Rhizobium* inoculant, believing that indigenous or previously introduced populations of rhizobia are sufficient to nodulate the plants. Scientific studies show alfalfa to be an excellent N-fixing crop, capable of fixing up to 200kg N/ha. It is assumed that the high productivity of this crop in producer's fields is due to N fixation, however, alfalfa also is extremely efficient at extracting inorganic N from soil solution (Blumenthal and Russelle, 1996) and when it is intercropped it is a strong competitor for soil N (Tomm et al., 1995). Strains of alfalfa have been selected for their efficient nitrate uptake characteristics, and have been used to "clean-up" nitrate fertilizer spills on agricultural land (Russelle et al., 2001). Biological nitrogen fixation continues when extremely high levels of nitrogen fertilizer is applied (Lamb et al., 1995). When 840 kg N/ha was applied, a first production year stand of alfalfa obtained 20-25% of its N from biological nitrogen fixation. The idea that alfalfa cannot benefit through both biological nitrogen fixation and N fertilization needs to be challenged.

Studies performed in the 1980's in northern Alberta, looked at post-emergent inoculation of alfalfa (Rice and Olsen, 1991; Rice, 1987). Granular rhizobial inoculants were placed beside the seed row the year after establishment. Other treatments included powdered seed applied inoculants, granular inoculants placed with the seed at seeding, granular inoculants placed below the seed at seeding, and liquid formulations placed both at seeding and post-emergently. None of the treatments showed any difference in yield over a three year period. Nodule numbers were higher for inoculated plants than controls, but all nodule numbers per plant were low and declined substantially with each year of the study. By the third year an average range of 0 to 2.4 nodules per plant was recovered from any of the treatments. Although not a conclusion of the authors the lack of yield response may have been because some or all of the plants were extracting soil N rather than fixing atmospheric N. Inoculation may have only been effective in the first year or two of establishment. Plant nitrogen contents were not assessed in the study. Furthermore, the lack of response with post-emergent inoculation may be due to damage caused by the post-emergent application itself rather than ineffective inoculation. There was no description in the report of the equipment used to place inoculant post-

emergently. Low disturbance disc seeders may improve the probability of successful post-emergent inoculation.

Annual applications of fertilizer to forages is recommended in the Forage Crop Production Guide 2003 (SAF, 2003), but in practice very few forage producers follow this advice. More commonly fertilization is utilized as a strategy for rejuvenation of forage grasses but is rarely performed on alfalfa. Alfalfa tends to be terminated once stand condition deteriorates. Nitrogen fertilization is not recommended for forage legumes. Inorganic soil N reduces the effectiveness of N fixation in legumes. It is assumed that because alfalfa fixes N biologically, N supply to the crop is not limiting. However, direct evidence of this is lacking. Little is known about how soil fertility changes under an aging stand. Furthermore, nodules are extremely difficult to find on the roots of an aging stand. Whether this is because they don't persist long term or is simply because they are difficult to harvest because of the depth of rooting is uncertain. In addition, we know little about the long-term survival and the long-term effectiveness of introduced rhizobia that may survive in the soils. The assumption that a reduction in N fertility is not the cause of stand degeneration may not be substantiated. Even if the nodules do persist, the addition of N fertilizer may invigorate the stand, enabling it to tiller more and reestablish in the stand. Nitrogen fertilizer does not permanently impair biological N fixation but simply delays it until low N conditions return. Another reason for not applying N fertilizers to alfalfa is that N fertilizers may increase the competitiveness of grasses in the stand. This is a concern in mixed stands. However, due to the extremely competitive nature of alfalfa for extracting inorganic N from the soil, this may not be as much of a problem as was initially thought. In pure stands, a combination of a herbicide to control grassy weeds and an N-fertilizer application might prove to be the most effective strategy for rejuvenating an aging stand.

Phosphorus fertilization is recommended for alfalfa stands which are more than 4 years old, and K and S are recommended for coarse textured soils and gray-wooded soils. Levels of plant available P can be extremely low in Saskatchewan soils and hence the potential for P deficiency to develop in forage crops is high. Phosphorus is a difficult nutrient to apply as an amendment because of its extremely low solubility. Strategies and/or products that increase the solubilization of soil phosphorus or fertilizer P may

prove effective in alfalfa. Due to practical limitations, this proposal deals only with N and P fertility strategies because these two nutrients are expected to have the largest effect on yield and microbial inoculants are available for increasing availability of both of these nutrients.

Aside from a few studies examining post-emergence inoculation of alfalfa stands in the year after establishment, no studies could be found looking at rhizobial inoculant application as a means of rejuvenating aging stands. Unlike N fertilization, successful re-inoculation of alfalfa would not stimulate grassy weed growth nor forage grasses in mixed stands. In addition, the commercialization of the P-solubilizing fungus, *Penicillium bilaiae* (JumpStart® Philom Bios, Inc., Saskatoon, SK) provides an opportunity for improving P fertility biologically. If successful, both rhizobial and *P. bilaiae* inoculation could provide a rejuvenation strategy for organic producers who do not have fertilization available to them as an option.

The overall objective of this study is to examine fertilization and microbial inoculation as a means of rejuvenating aging alfalfa stands. N and P fertilizers were examined, as well as inoculation with rhizobia and *P. bilaiae* inoculants. In addition, post-emergent inoculation of alfalfa with granular rhizobial and *P. bilaiae* inoculants in the year after establishment was examined. This study was intended as a broad-scope study, to identify general fertility responses of aging alfalfa fields and was not intended to identify specific application rates of the products.

c) Methods

Rejuvenation of aging alfalfa stands:

<u>Trial Establishment:</u> Three fields with aging alfalfa stands were chosen for study. The fields are located near Smeaton, Star City, and Crooked River, Saskatchewan. Soils from the Star City and Smeaton sites are light-textured Shellbrook loam soils. At the Crooked River site, the soil is a heavier textured Tisdale clay loam soil. Fields were sampled in early spring prior to treatments being applied. The fields were selected on the basis of soil tests; all fields had phosphorus test results in the marginal to deficient range and nitrogen test results in the marginal range (Table 1). All of the fields were showing

Table 1. Soil physico-chemical properties and nutrient availability at the three study sites in May 2004. Mean value of six replicates with standard deviation presented in parenthesis.

	Star City			Smeaton			Crooked River		
	Depth (cm)								
	0 to 15	**15 to 30**	**30 to 60**	**0 to 15**	**15 to 30**	**30 to 60**	**0 to 15**	**15 to 30**	**30 to 60**
Texture class[1]	*L*	*L-CL*	*L*	*L*	*L-CL*	*CL*	*CL*	*CL*	*C*
ρ_b (g cm^{-3})	1.52 (0.05)	1.46 (0.01)	1.40 (0.12)[2]	1.36 (0.11)	1.52 (0.06)	1.58 (0.13)[2]	1.34 (0.07)	1.45 (0.03)	1.38 (0.10)[2]
pH	6.8 (0.4)	7.3 (0.4)	7.9 (0.6)	5.8 (0.3)	6.7 (0.3)	7.5 (0.5)	6.4 (0.1)	7.3 (0.2)	7.9 (0.2)
EC (dS m^{-1})[3]	0.20 (0.10)	0.25 (0.10)	0.27 (0.10)	0.20 (0.08)	0.17 (0.04)	0.37 (0.16)	0.24 (0.03)	0.25 (0.03)	0.43 (0.04)
Total C (%)	1.80 (0.19)	1.11 (0.48)	1.95 (0.85)	2.52 (0.93)	1.01 (0.36)	0.94 (0.36)	2.60 (0.39)	1.21 (0.19)	1.59 (0.47)
Organic C (%)	1.36 (0.20)	0.61 (0.24)	0.48 (0.22)	2.21 (0.83)	0.67 (0.36)	0.40 (0.15)	2.26 (0.26)	0.81 (0.12)	0.60 (0.12)
Total N (mg g^{-1})	0.14 (0.01)	0.07 (0.02)	0.05 (0.02)	0.20 (0.08)	0.08 (0.03)	0.05 (0.02)	0.22 (0.03)	0.11 (0.01)	0.09 (0.01)
inorganic N (μg g^{-1})	7.9 (2.0)	7.1 (1.3)	5.9 (2.5)	10.8 (3.3)	6.1 (2.3)	6.5 (4.7)	6.9 (1.4)	6.2 (1.1)	7.3 (1.4)
P (μg g^{-1})	18.2 (8.5)	25.1 (7.2)	12.1 (5.6)	9.8 (4.1)	14.4 (3.6)	15.7 (4.0)	14.0 (4.0)	19.9 (2.7)	15.7 (2.0)
K (μg g^{-1})	99 (18)	107 (20)	86 (24)	103 (30)	98 (26)	113 (31)	171 (31)	165 (29)	152 (31)
Total S (mg g^{-1})	0.014 (0.01)	0.009 (0.00)	0.007 (0.00)	0.022 (0.01)	0.014 (0.01)	0.009 (0.00)	0.021 (0.01)	0.013 (0.00)	0.008 (0.00)
SO_4^{2-}-S (μg g^{-1})	4.0 (1.2)	2.5 (0.7)	2.3 (0.5)	11.4 (12.2)	4.4 (2.2)	4.8 (3.9)	8.3 (1.1)	4.7 (0.8)	4.8 (2.1)

[1] Loam (L), Clay (C), Clay-loam (CL)

[2] 30- to 45-cm depth increment.

[3] EC values corrected to saturated paste equivalent EC values.

decreased productivity (as reported by the farmer) that was suspected by the farmer to be the result of fertility decline rather than weed invasion.

Treatments (Table 2) were applied in early May, 2004 by a graduate student, and Philom Bios technicians.

All of the microbial inoculants were prepared by Philom Bios as granular formulations. The strain of *Sinorhizobium meliloti* in the rhizobial inoculant was NRG-34 (N-Prove, Philom Bios Inc.). The inoculant contained approximately 6.04 x 10^8 cell of *S. meliloti* g^{-1} inoculant. The *P. bilaiae* inoculant contained the PB-50 strain (JumpStart, Philom Bios Inc.) and had approximately 2.2 x 10^6 fungal spores g^{-1} inoculant. The rhizobia plus *P.bilaiae* treatment was the TagTeam product manufactured by Philom Bios Inc. The inoculants were applied using a modified small-plot seeder supplied by Philom Bios. The seeder was equipped with narrow-row disc openers with on-row press wheel packing (Flexi-Coil). The seeder had five rows spaced 30 cm apart. Inoculants were placed 4 cm deep. Soil and residue disturbance was minimal although some damage was evident (Plate 1 – back of report with project photographs).

The granular carrier used in the manufacture of the rhizobial and *P. bilaiae* inoculants is gypsum ($CaSO_4 . 2H_2O$), which contains 18.6% S by weight. Application of the high rate of inoculants (10X = 40 kg ha^{-1}) results in the placement of 7.4 kg S ha^{-1}. To avoid the confounding effect of this S application to the inoculant treatment plots, all of the plots except the untreated control, and mechanically disturbed control received the equivalent of 40 kg ha^{-1} total gypsum. In addition, a control receiving 40 kg ha^{-1} gypsum was included.

Triple superphosphate was banded using the same small-plot seeder used for the inoculant applications. N and S fertilizers were hand- broadcast evenly across the surface of the treatment plots.

The experimental design was a randomized complete block with six replicates. Individual plots were 10 m by 1.5 m. A 6 m pathway separated blocks.

Table 2. Fertilizers and microbial inoculants applied to aging alfalfa stands.

Treatment	**Application method**
Nitrogen :	
Ammonium nitrate (100 kg N ha^{-1})	Broadcast
Urea (100 kg N ha^{-1})	Broadcast
Urea & Agrotain (100 kg N ha^{-1})	Broadcast
Slow release urea[1] (100 kg N ha^{-1})	Broadcast
Rhizobia (1X)[2]	Banded
Rhizobia (10X)[2]	Banded
Phosphorus :	
Triple superphosphate (40 kg P ha^{-1})	Banded
P. bilaiae (10X)[2]	Banded
P. bilaiae (10X)[2] & triple superphosphate (20 kg P ha^{-1})	Banded
Nitrogen and Phosphorus:	
Rhizobia and *P. bilaiae* (10X)[2]	Banded
Sulphur:	
Ammonium sulphate	Banded
Controls:	
Undisturbed	
Mechanically disturbed	Banded
Gypsum	Banded

[1]Agrium. slow release urea

[2]Applied at the recommended (1X) or ten times the recommended (10X) application rate – recommended application rate is 4.0 kg ha^{-1}.

Sampling and measurements: Soil samples were collected in the spring of 2004 from the undisturbed control plots. Composite samples from three randomly selected points in each plot were collected at 0- to 15-cm, 15- to 30-cm, and 30- to 60- cm depths, using a Dutch auger with a 6-cm diameter. Soils were air-dried at 40° C and ground to pass through a 2-mm sieve.

Soils were analysed for pH (Hendershot et al., 1993) and electrical conductivity (EC) (Janzen, 1993) using a 1:2 (soil:water) extraction. Soil texture was determined using the hydrometer method (Gee and Bauder, 1986). Percentage of organic carbon (OC) in soils was measured using a LECO carbon analyser. Total soil C, N and S were measured with a LECO CNS 2000 combustion analyser. Exchangeable soil NO_3^- and NH_4^+ were extracted with a 2.0 *M* KCl solution (10:1) (Maynard and Kalra, 1993) and analysed colorimetrically with autoanalyser. Sulphate was extracted from the soils with a $CaCl_2$ solution (2:1) and analysed colorimetrically on an autoanalyser. P and K were extracted using the modified Kelowna method (Qian et al., 1994); the K was measured with flame emission spectrometry and the P colorimetrically with an autoanalyse. Bulk density was determined by taking three random soil cores (1.95 cm diameter) from each plot and separating into 0- to 15-cm, 15- to 30-cm, 30- to 60-cm depths. Soil cores were dried at 100° C, weighed and bulk density calculated for each soil volume.

Growing season temperature and precipitation data were obtained from the Environment Canada weather database (Environment Canada, 2005). The Tisdale weather station was used for the Star City and Crooked River sites and the Nipawin weather station for the Smeaton site.

Stand counts (plants m^{-2}) were performed approximately one month after the establishment of the trials. All of the control plots and four treatment plots were measured to quantify the degree of damage from mechanically disturbing the plots. Numbers of individual stems in a 0.25 m^2 quadrant were counted.

In 2004 and 2005, plants were sampled at an early vegetative stage from plots receiving the P-treatments. Responses to P fertilizers and inoculants are frequently most apparent early in the growing season. Cumulative samples of two randomly place 0.25

m^2 quadrants were taken from each plot. Plants were clipped at approximately 4 cm above the soil surface. Samples were dried at 40° C until a constant mass was reached.

Root sampling was attempted in 2004 and 2005. However, we were unable to excavate any roots with nodules due in part to the dry soils, but also because the nodules seemed to be very deep in the soil.

In 2004 two cuts were taken from the Smeaton and Crooked River sites; one in late June and a second 6 to 8 weeks later. The Star City site did not have sufficient regrowth to remove a second cut. In 2005, two cuts were taken from Smeaton site. Excessive rain in August prevented a second sampling from Crooked River. Star City did not have sufficient regrowth in 2005 to warrant another cut. All sites were sampled only once in the 2006 season. Randomly selected m^2 quadrants were hand-harvested with a sickle at a 4 cm height. Samples were dried in a ventilated hot box at approximately 38° C until constant mass was attained. After hand sampling was complete the entire biomass from the site was cut and removed.

Plant samples were ground (1-mm mesh). Subsamples were acid digested following standard procedures (Thomas et al., 1967). Concentrations of N and P in the digested solutions were determined colorimetrically using an autoanalyser. Samples from the undisturbed controls in 2004 were analyzed for calcium, magnesium, potassium, iron, manganese, copper, zinc, and boron, to get a base level of nutritional health of the stands.

Plant S concentration was analysed in the control and ammonium sulphate treated plots at Crooked River and Smeaton, and the control, gypsum and ammonium sulphate treated plots at Star City. Star City was the most responsive of all of the sites to the treatments and especially the S treatments.

Quantification of *S. meliloti* populations in the soil: Soils from the three sites were collected from the *Rhizobia* (1X); *Rhizobia* (10X) and control plots (all established in 2004) in early summer of 2005. The objective was to quantify "natural' populations of Rhizobia in the control plots and determine if inoculation with S. meliloti increased these populations after one season. The soils were subjected to a Most Probable Number (MPN) assay, whereby a series of dilutions of the soil extract are used to inoculate the

root systems of alfalfa seedlings grown in near sterile conditions. Nodule numbers are counted throughout the growth period and used to mathematically determine an estimate of the numbers of viable rhizobia in the soil. Only those rhizobia that can (and do) initiate pink active nodules are scored.

Delayed inoculation study:

Two field sites were established near Aberdeen, SK and a third site near Arborfield, SK. Field soils were sampled in early spring. All sites had deficient P values and marginal N values (Table 3). Because of the previous two drought years, it was difficult to locate fields extremely low in N. All of the plots received a blanket treatment of potassium sulphate fertilizer (25 kg S ha^{-1}). Year of seeding treatments were seeded in May, 2004 (Table 4). Five additional plots per replicate block were established with alfalfa and the blanket treatment of potassium sulphate. The following spring (May 2005) the delayed treatments were applied to these plots (Table 4) by banding in the appropriate inoculant or inoculant/fertilizer combination. Alfalfa (*Medicago sativa* cv Algonkwin) was seeded at a rate of 7.8 kg/ha.

One of the Aberdeen sites was severely flooded in the spring of 2005 (See Plate 1) and the site had to be ploughed under in June of 2005. Thus, we had only two of three original sites remaining for this project.

^{15}N labeled ammonium nitrate fertilizer was applied in 2005 to m^2 subplots within each main plot to estimate biological N_2 fixation. The subplots were sampled just prior to the biomass samples being harvested. Approximately five stems were randomly sampled from the subplot, bagged, dried, ground and analysed for ^{15}N content. Biological N fixation was estimated using the isotope dilution method (Hardarson and Danso, 1990).

Table 3. Soil characteristics for the delayed inoculation sites prior to seeding in 2004. Values are for a composite of 10 soil samples (0 to 30 cm) randomly sampled from the field sites.

Site	P ($\mu g\ g^{-1}$)	SO_4-S ($\mu g\ g^{-1}$)	K ($\mu g\ g^{-1}$)	Inorg. N ($\mu g\ g^{-1}$)	pH	EC (dS m^{-1})
Aberdeen	29	36	>1020	41	7.8	0.3
Arborfield	38	47	301	15	7.2	0.2

Table 4. Description of treatments applied in the delayed inoculation study. Year of seeding treatments had inoculants applied at the time of seeding. Delayed inoculation treatments were banded into a 1 year old standing crop.

Year of seeding treatments	Delayed treatments
Control: Uninoculated seed, no fertilizer	Control: mechanical disturbance
Uninoculated seed, recommended rate of N[1]	-
Uninoculated seed, recommended rate of P[2]	-
Uninoculated seed, recommended rate of N and P	-
Seed inoculated with rhizobia[3]	Rhizobia banded
Seed inoculated with *P. bilaiae*[4]	*P. bilaiae* banded
Seed inoculated with rhizobia and *P. bilaiae*	Rhizobia and *P. bilaiae* banded
Seed inoculated with *P. bilaiae* on soil fertilized with ½ rate of P.	*P. bilaiae* and ½ rate P banded

[1]20 kg N h^{a-1} side-banded
[2]20 kg P ha^{-1} seed-placed
[3]4 hg ha^{-1}
[4]4 kg ha-[1]

Statistical Analyses:

Statistical analyses were conducted using SPSS version 14.0. One way analysis of variance was run for each harvest at the three sites separately. Fisher protected LSD was calculated for each variable at a probability level of 0.10. Due to the complexity of the treatments, orthagonal contrasts were developed to examine specific treatment comparisons in more detail. Because of the highly variable nature of the field studies, significance of orthagonal contrasts is reported for p-values of 0.2, 0.1 and 0.05.

d) **Results:**

Rejuvenation of aging alfalfa stands:

Stand counts:

Mechanical disturbance from the disc-banding operation caused very little disturbance to the stands and did not affect ($P \leq 0.10$) the stand densities at either the Star City or Smeaton sites (Table 5). At Crooked River, a two-tailed, paired sample Student's t-test revealed that the stand count was significantly reduced ($P = 0.03$) from a mean of 36 plants m^{-2} in the control plots to a mean of 32 plants m^{-2} in the M.D. plots. However, counts at this site were comparable to the other two sites. The large variability with these stand counts was due to the difficulty associated with determining which stems were individual plants and which stems were coming from the same crown.

Table 5. Mean alfalfa stand counts in May 2004 from mechanically disturbed plots and control plots at the three field sites.

	Smeaton	Star City	Crooked River
		------------------ *plants m*$^{-2}$ ------------------	
M.D.[2]	33 (14)	[1]30 (20)	32 (15)
Control	28 (16)	26 (4)	36 (14)
		------------------ *P-value*[3] ------------------	
	0.39	0.61	0.03

[1]Mean of 6 replicates with standard deviation in parenthesis.
[2]Mechanical Disturbance
[3] Level of probability that means are not significantly different according to the two-tailed, paired sample Student's *t*-test.

Early spring Phosphorus Availability:

Crop responses to P amendments are most commonly observed in cool, wet spring conditions when P availability is most limiting. In both 2004 and 2005, although temperatures were below the 15 year average, precipitation was low and soil moisture conditions dry (data not shown). None of the fertilizer or inoculant treatments had any effect on P uptake by alfalfa plants in the early spring of either year (Tables 6 & 7).

Table 6: Phosphorus uptake by alfalfa harvested in early spring at three sites in Saskatchewan. Treatments were applied to mature stands in the spring of 2004. Tissue samples were collected 6 weeks after treatments were applied in 2004, and 6 weeks after spring plant growth initiated in 2005.

Treatment	*Smeaton*		*Star City*		*Cr. River*	
	2004	**2005**	**2004**	**2005**	**2004**	**2005**
TSP[1]	4.0[4]	4.0	4.3	4.2	3.7	4.5
P. bilaiae	3.6	3.6	4.4	4.2	3.8	4.3
P. bilaiae + TSP	3.7	3.5	4.5	4.4	3.4	4.1
Rhizobia + *P. bilaiae*	3.5	3.7	4.7	4.2	3.7	4.1
MD[2]	3.7	3.8	4.3	4.2	3.3	4.0
MD + granule[3]	3.7	3.6	4.6	4.2	3.5	3.9
Control	3.7	3.5	4.6	4.4	3.6	4.0

[1]TSP=triple superphosphate fertilizer
[2]MD=mechanical disturbance
[3]granule is the gypsum granule used as inoculant carriers
[4]Values within a column, followed by different letters are significantly different according to lsd ($p \leq 0.05$). Where no letters appear, differences are not significant ($p > 0.05$).

Either P was not limiting, or environmental conditions were not conducive to enhanced P availability for uptake.

Productivity and Nutrient Acquisition – Smeaton:

In the treatment year (2004) application of the N-fertilizers increased tissue N concentrations and N acquisition by the alfalfa plants compared to the controls (Table 7). In addition, N-fertilization increased tissue N concentration and N acquisition over that of the rhizobial inoculants. Biomass production was also higher in the N-fertilizer plots compared to those inoculated with rhizobia. N fertilizers that are immediately available (urea and ammonium nitrate) were superior to the slow release formulations (Urea + Agrotain and ESN) at this first cut harvest. Inoculation with rhizobia did not affect N status nor productivity compared to the controls. None of the P or S treatments had any effect on productivity or nutrient acquisition.

Mechanically disturbing the plots increased N acquisition by the alfalfa plants compared to the undisturbed controls, by the second cut of the season (Table 8). Soil disturbance can enhance N mineralization by incorporating organic matter into the soil, making it more available for microbial degradation. In addition, soil disturbance aerates soil and can increase soil temperatures, both of which can stimulate microbial activity. Clearly at this site, working the soil provided more benefit than the risk of damage to the alfalfa plants.

The benefit of the N fertilizers to the N status of the plants was lost by the second harvest (Table 8). Tissue N concentrations were lower in the N fertilized plants than the controls but was due to the dilution of the N in the larger biomass produced by the N fertilized plants. On a per land basis, N acquisition was similar between N fertilized and controls. The advantage of the readily available N fertilizers over the slow release fertilizers was maintained throughout the season.

At this second harvest there was also some benefit in terms of N acquisition provided by adding S to the system. Taken together, the S fertilizer and the gypsum granules increased N

Table 7. Productivity and nutrient status of mature alfalfa harvested mid-season in 2004 (first of two cuts) at the Smeaton site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Urea	1	2720	30.6	83.3	2.2	6.0
Ammon. nitrate	2	2703	37.4	99.2	2.3	6.2
Urea + Agro.	3	2258	37.2	86.8	2.1	5.0
ESN	4	2513	32.5	88.2	2.3	6.2
Ammon. sulphate	5	2163	34.0	82.5	2.4	5.9
Rhizobia (1X)	6	1951	32.7	73.8	2.3	5.2
Rhizobia (10X)	7	2393	32.5	81.9	2.4	6.0
TSP	8	2601	31.8	83.1	2.6	6.9
P. bilaiae	9	2260	33.7	81.0	2.4	5.6
P. bilaiae + ½ P	10	1896	33.8	78.2	2.5	5.7
Rhiz. + *P. bilaiae*	11	2308	30.8	72.1	2.5	5.9
MD	12	2253	31.0	76.9	2.4	5.7
MD + granule	13	2310	31.1	70.0	2.3	5.2
Undisturbed	14	2068	31.2	63.1	2.4	4.9
LSD (0.05)		964	24.2	5.5	0.3	1.7
Contrasts	*Trt compared*	***Difference between means***[1]				
Disturbed – undisturbed controls	14-12,13	227	-0.7	20.8	-0.2	1.0
No granule – granule	12-13	-57	-0.1	6.9	0.1	0.6
N fertilizer – control	1,2,3,4-13	955	13.4**	77.4***	-0.2	2.8
N fertilizer – rhizobia	1,2,3,4-6,7	1505**	7.2*	46.1**	-0.4	1.0
Rhizobia – control	6,7-13	-275	3.1	15.7	0.1	0.9
P fertilizer – *P. bilaiae*	9-8	341	-1.8	2.1	0.3**	1.3**
N fertilizer – slow release N fertilizer	1,2 -3,4	651*	-1.7	7.6	0.1	1.0
S fertilizer – control	5-12	-90	3.1*	5.6	0.1	0.2
S – control	5,13-12	-33	3.1	-1.3	0.1	-0.3
Rhiz. – Rhiz. + *P.b.*	7-11	-85	-1.6	-9.7	0.1	-0.1
P.b. – Rhiz. + *P.b.*	9-11	48.3	-8.8	-2.8	0.1	0.2

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.
[1] grams of nutrient per kg of above ground biomass
[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

Table 8. Productivity and nutrient status of mature alfalfa harvested late-season in 2004 (second of two cuts) at the Smeaton site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Urea	1	2715	29.7	81.4	2.1	5.7
Ammon. nitrate	2	2738	23.5	59.7	1.7	4.4
Urea + Agro.	3	2701	30.3	78.6	2.0	5.0
ESN	4	2818	28.8	82.8	1.9	5.6
Ammon. sulphate	5	2930	27.5	79.2	1.8	5.2
Rhizobia (1X)	6	2580	27.9	71.3	1.9	4.8
Rhizobia (10X)	7	2763	27.2	77.4	1.9	5.3
TSP	8	2990	29.7	83.3	2.2	6.1
P. bilaiae	9	2810	30.5	82.8	2.0	5.5
P. bilaiae + ½ P	10	2451	25.6	70.6	1.8	4.9
Rhiz. + *P. bilaiae*	11	2600	29.7	72.1	2.0	4.9
MD	12	2581	27.6	71.1	1.8	4.7
MD + granule	13	2545	30.2	80.8	2.0	5.4
Undisturbed	14	2457	27.9	64.3	2.0	4.6
LSD (0.05)		612	14.5	5.0	0.5	1.5
Contrasts	*Trt compared*			*Difference between means[1]*		
Disturbed – undisturbed controls	14-12,13	213	2.1	23.2***	-0.1	1.0
No granule – granule	12-13	37	-2.6	-9.7*	-0.1	-0.6
N fertilizer – control	1,2,3,4-13	793*	-8.5*	-20.5	-0.2	-0.7
N fertilizer – rhizobia	1,2,3,4-6,7	287	2.2	5.3	0.2	0.6
Rhizobia – control	6,7-13	253	-5.4*	-12.9	-0.2	-0.6
P fertilizer – *P. bilaiae*	9-8	178	-0.8	0.5	0.2	0.6
N fertilizer – slow release N fertilizer	1,2 -3,4	-67	-5.9**	-20.3***	-0.1	-0.5
S fertilizer – control	5-12	348*	-0.1	8.1	0.0	0.5
S – control	5,13-12	311	2.6	17.8*	0.1	1.1
Rhiz. – Rhiz. + *P.b.*	7-11	-163	2.5	-5.3	0.2	-0.4
P.b. – Rhiz. + *P.b.*	9-11	-211	-107	-0.8*	0.0	-0.6

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

acquisition. Because of its low solubility and low mobility, it is not unusual to see the effect of S applications late in the season, but not early.

The advantage conferred to the tissue N contents and N acquisition by mechanically disturbing the plots continued into the second year of the study (Table 9) but had disappeared by the second harvest of year 2 (Table 10). These mechanically disturbed plots also had higher P uptake. In 2005 (year 2) the largest effect on biomass production, and N acquisition was attributable to the S containing amendments. Both the ammonium sulphate fertilizer and the gypsum granules increased productivity and N acquisition. Inoculation with rhizobia increased tissue N contents at the first harvest compared to N fertilization (Table 9) but did not translate into higher productivity nor higher N acquisition. In addition, this advantage of the rhizobia inoculated plots was transient and did not persist to the second harvest. However, when comparing the rhizobia and *P. bilaiae* applied individually and the dual application of the organisms, both organisms functioned better alone than when in combination (Table 10). Rhizobia alone increased tissue N concentrations and *P. bilaiae* alone increased N acquisition compared to the dual application. Rhizobia are sensitive to pH fluctuations, and unless they are selected from acidic soils, tend not to grow or function well under acidic conditions. *Pencillium bilaiae* function by producing and excreting organic acids into the rhizosphere making P more soluble in these acidified areas. Perhaps the close proximity of the two organisms in the dual application makes soil conditions unfavorable for the rhizobia.

The final year of the study (2006) indicated that the majority of the effects of the amendments had subsided by this time (Table 11). There was still a slight advantage of the immediately available fertilizers compared to the slow release fertilizers in tissue N concentrations, but this did not translate into increased productivity or total N acquisition.

Table 9. Productivity and nutrient status of mature alfalfa harvested mid-season in 2005 (first of two cuts) at the Smeaton site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Urea	1	3135	24.9	79.9	2.0	6.4
Ammon. nitrate	2	3130	24.9	77.1	2.1	6.6
Urea + Agro.	3	3062	25.5	79.2	2.1	6.5
ESN	4	3398	24.8	81.4	2.1	7.1
Ammon. sulphate	5	3367	27.0	91.7	2.1	7.1
Rhizobia (1X)	6	2913	27.6	86.3	2.2	7.0
Rhizobia (10X)	7	3268	25.8	81.9	2.1	6.8
TSP	8	2750	25.3	71.0	2.2	6.3
P. bilaiae	9	2855	26.4	75.2	2.3	6.6
P. bilaiae + ½ P	10	2760	25.6	77.5	2.1	6.4
Rhiz. + *P. bilaiae*	11	2905	26.3	78.3	2.3	6.9
MD	12	2898	26.2	72.6	2.2	6.2
MD + granule	13	3308	26.4	91.7	2.1	7.4
Undisturbed	14	2811	24.3	67.0	2.1	5.8
LSD (0.05)		919	22.8	2.7	0.3	2.1
Contrasts	*Trt compared*			*Difference between means*[1]		
Disturbed – undisturbed controls	14-12,13	-17	3.9**	30.2**	0.2	2.0*
No granule – granule	12-13	-410*	-0.2	-19.0*	0.1	-1.2*
N fertilizer – control	1,2,3,4-13	-508	-5.4*	-18.9*	-0.1	-3.0
N fertilizer – rhizobia	1,2,3,4-6,7	362	-6.9***	-18.8	-0.3	-0.9
Rhizobia – control	6,7-13	-435	0.8	-15.0	0.1	-1.1
P fertilizer – *P. bilaiae*	9-8	-105	-1.1	-4.3	-0.1	-0.4
N fertilizer – slow release N fertilizer	1,2 -3,4	-195	-0.4	-3.6	-0.1	-0.6
S fertilizer – control	5-12	468*	0.8	19.1*	-0.2	0.9
S – control	5,13-12	878*	1.0	38.1**	-0.3	2.0*
Rhiz. – Rhiz. + *P.b.*	7-11	-363	0.5	-3.6*	0.2	0.1
P.b. – Rhiz. + *P.b.*	9-11	50	3.1	0.1	0.0	0.2

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

Table 10. Productivity and nutrient status of mature alfalfa harvested late-season in 2005 (second of two cuts) at the Smeaton site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Urea	1	2087		65.6	1.9	4.0
Ammon. nitrate	2	1875	31.4	50.6	1.9	3.3
Urea + Agro.	3	1548	29.3	46.0	1.9	2.9
ESN	4	1813	30.0	54.3	1.9	3.5
Ammon. sulphate	5	1825	30.6	56.0	1.9	3.4
Rhizobia (1X)	6	1678	32.1	54.9	1.7	3.2
Rhizobia (10X)	7	1901	29.4	53.3	1.8	3.3
TSP	8	1675	29.3	54.1	2.1	3.5
P. bilaiae	9	1783	32.4	53.8	1.9	3.5
P. bilaiae + ½ P	10	1531	29.9	59.3	2.0	3.7
Rhiz. + *P. bilaiae*	11	1895	31.6	55.8	2.1	3.5
MD	12	1803	33.3	51.4	1.8	3.3
MD + granule	13	1878	27.3	53.9	1.9	3.4
Undisturbed	14	1760	29.3	58.0	1.8	3.6
LSD (0.05)		601	20.6	3.0	0.2	1.3
Contrasts	*Trt compared*	*Difference between means[1]*				
Disturbed – undisturbed controls	14-12,13	-238*	-2.2	-10.7	0.0	-0.4
No granule – granule	12-13	-75	-2.0*	-2.5	-0.1	-0.1
N fertilizer – control	1,2,3,4-13	-190	4.0	1.1	0.1	-0.1
N fertilizer – rhizobia	1,2,3,4-6,7	163	3.8	0.2	0.6***	0.7
Rhizobia – control	6,7-13	-177	0.1	0.4	-0.2*	0.4
P fertilizer – *P. bilaiae*	9-8	-108	2.5**	0.3	0.1*	0.0
N fertilizer – slow release N fertilizer	1,2 -3,4	600**	0.1	15.8*	0.0**	0.9
S fertilizer – control	5-12	21	4.8***	4.7	0.2*	0.0
S – control	5,13-12	97	6.8***	7.1	0.3**	0.1
Rhiz. – Rhiz. + *P.b.*	7-11	-7	4.0***	2.5	0.3	0.2
P.b. – Rhiz. + *P.b.*	9-11	111	2.0	3.4***	0.1*	0.0

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

Table 11. Productivity and nutrient status of mature alfalfa harvested in 2006 (a single cut was harvested) at the Smeaton site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass ($kg\ ha^{-1}$)	tissue N[1] ($g\ kg^{-1}$)	N acquis.[2] ($kg\ ha^{-1}$)	tissue P[1] ($g\ kg^{-1}$)	P uptake ($kg\ ha^{-1}$)
Urea	1	1085	160.2	163.0	15.0	16.0
Ammon. nitrate	2	1084	182.8	200.3	15.4	16.7
Urea + Agro.	3	990	187.2	178.5	15.6	14.9
ESN	4	1173	181.7	149.2	15.6	15.3
Ammon. sulphate	5	993	175.9	175.8	16.0	16.1
Rhizobia (1X)	6	845	176.7	147.7	15.4	13.2
Rhizobia (10X)	7	1316	185.6	250.5	14.9	19.8
TSP	8	1039	179.3	189.1	14.2	13.0
P. bilaiae	9	1074	173.8	186.0	15.3	16.3
P. bilaiae + ½ P	10	1105	154.7	169.6	14.9	16.9
Rhiz. + *P. bilaiae*	11	1195	188.7	228.2	14.7	17.2
MD	12	1303	176.6	228.2	14.6	18.9
MD + granule	13	1095	173.5	182.2	13.6	14.3
Undisturbed	14	1109	173.8	196.3	14.9	16.8
LSD (0.05)		856	157.8	37.6	3.3	12.4
Contrasts	*Trt compared*			*Difference between means*[1]		
Disturbed – undisturbed controls	14-12,13	179	2.4	17.7	-1.5	-1.5
No granule – granule	12-13	208	3.0	46.0	1.0	1.0
N fertilizer – control	1,2,3,4-13	-49	17.8	-37.8	7.1**	7.1
N fertilizer – rhizobia	1,2,3,4-6,7	12	-12.9	-105.6	0.9	0.9
Rhizobia – control	6,7-13	-30	15.3	33.9	3.1*	3.1
P fertilizer – *P. bilaiae*	9-8	-36	5.5	3.02	-1.1	-1.1
N fertilizer – slow release N fertilizer	1,2 -3,4	5	-25.9*	35.6	-0.8	-0.8
S fertilizer – control	5-12	-310	-0.6	-52.4	1.5*	1.5
S – control	5,13-12	-518	-3.7	-98.4	0.5	0.5
Rhiz. – Rhiz. + *P.b.*	7-11	-120	3.0	-22.4	-0.2	-0.2
P.b. – Rhiz. + *P.b.*	9-11	121	42.1	14.9	-0.6	1.0

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biom3.1ass per hectare of land and includes both N taken up from the soil and biological N fixation

Tissue S concentration and S uptake was examined in the control and ammonium sulphate fertilized alfalfa (Table 12) to determine if S was limiting at this site. While S fertilization stimulated N uptake and biomass production, tissue S concentrations and uptake were not significantly different between the fertilized and control plots. Sulphur concentrations in alfalfa typically are in the 2.5 to 5 mg g^{-1} range depending on the stage of growth (Rominger et al., 1975).

Table 12. Mean S concentration and uptake (± standard error) in alfalfa amended with ammonium sulphate fertilizer as well as a control for the first cut in 2004 at the Smeaton site (n=6).

Treatment[1]	**S-conc**	**S-uptake**[2]
	mg g^{-1}	*kg ha^{-1}*
Amm. Sulphate	4.9 (0.8)	10.8 (2.1)
Control	3.9 (0.5)	8.6 (1.9)
	-------------- *P-value*[3]	-------------
	0.44	0.56

[1] Refer to Table 2 for a complete description of treatments and application methods.
[2]S-uptake (kg ha^{-1})= S-conc (mg g^{-1}) x harvested biomass (g m^{-2}) x 10 000 m^2 ha^{-1} x 0.001 kg g^{-1}.
[3] Level of probability that means are not significantly different according to the two-tailed paired sample Student's *t*-test.

Productivity and Nutrient Acquisition – Star City:

The Star City site was the most responsive of the three sites to the treatments, in general. As was the case in Smeaton, mechanically disturbing the mature stand improved N acquisition, but unlike Smeaton also increased P uptake and overall productivity (Table 13). There was a strong response at this site to the S amendments (both S fertilizer and the gypsum granule), indicating that this site was S deficient. Biomass, tissue N concentrations, N acquisition and P uptake were all enhanced by the addition of S to the soil. The response to the single application of the S fertilizer and to a lesser extent the gypsum granule persisted through the second and third years of growth (Table 14 & 15). In 2004 the increase in biomass production due to the S fertilizer was 1007 % and the increase due to the gypsum was 66% (Table 13). In year two biomass increases were 168% and 20% for the fertilizer and gypsum respectively (Table 14), and in the third year a 98% increase in biomass was due to the S fertilizer (Table 15). The effect of the gypsum was no longer observable in year three. Because the ammonium sulphate was surface broadcast to the plots it would take time to work its way into the rooting profile of the alfalfa. Rainfall throughout the years translocated the S through the soil into the rooting zone, making it available for plant uptake.

Alfalfa production at this site also responded to P fertilization indicating a P deficiency. It is somewhat surprising that the application of *P. bilaiae* was not effective when P fertilization was, considering the relatively low concentrations of available P in the soil at this and the other sites. *Penicillium bilaiae* hyphae excrete organic acids into the rhizosphere. The release of H^+ ions lowers the pH which causes some bound P to be released into solution simply because of the acidifying conditions. In addition, specific organic acids (oxalic and citric acids) complex with calcium in the soil. This calcium normally adsorbs P_2O_5 ions, making them unavailable for plant uptake but when the organic acids complex with the calcium the P_2O_5 is released into soil solution. The *P. bilaiae* fungus does not work preferentially on either fertilizer P or native P supplies. However, because of the low solubility of P and its limited ability to move in the soil, it may simply be that the location of the *P. bilaiae* in the soil was not in close enough proximity to the active root system to be of any benefit with P uptake. In newly sowed crops the inoculants are

Table 13. Productivity and nutrient status of mature alfalfa harvested in 2004 (a single cut was harvested) at the Star City site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Urea	1	2415	30.5	74.0	3.0	7.1
Ammon. nitrate	2	2368	33.4	79.4	3.0	6.9
Urea + Agro.	3	2320	33.2	77.9	2.9	6.4
ESN	4	2247	31.7	71.3	3.1	7.0
Ammon. sulphate	5	2691	35.9	99.9	3.2	8.7
Rhizobia (1X)	6	2118	31.6	66.6	3.1	6.7
Rhizobia (10X)	7	2361	31.0	72.6	3.0	7.0
TSP	8	2715	33.1	90.4	3.2	8.6
P. bilaiae	9	1956	31.1	60.5	3.0	5.9
P. bilaiae + ½ P	10	2097	32.3	71.1	3.0	6.6
Rhiz. + *P. bilaiae*	11	2212	32.0	66.0	3.0	6.1
MD	12	1303	28.6	37.2	3.0	3.6
MD + granule	13	2168	29.2	62.9	3.1	6.6
Undisturbed	14	1080	29.2	31.4	3.2	3.3
LSD (0.10)		*1084*	*36.8*	*3.6*	*0.6*	*3.1*
Contrasts	*Trt compared*			*Difference between means*[1]		
Disturbed – undisturbed controls	14-12,13	1312**	-0.5	37.2*	-0.3	3.5**
No granule – granule	12-13	-865***	-0.5	-25.8**	0.0	-2.9***
N fertilizer – control	1,2,3,4-13	677	12.1***	51.0	-0.3	1.1
N fertilizer – rhizobia	1,2,3,4-6,7	390	3.6	24.2	-0.4	0.0
Rhizobia – control	6,7-13	143	4.3**	13.4	0.0	0.6
P fertilizer – *P. bilaiae*	9-8	758**	2.0*	30.0***	0.2	2.7***
N fertilizer – slow release N fertilizer	1,2 -3,4	217	-1.0	4.3	0.0	0.6
S fertilizer – control	5-12	1388***	7.3***	62.8***	0.2	5.1***
S – control	5,13-12	2253***	7.8***	88.5***	0.3	8.0***
Rhiz. – Rhiz. + *P.b.*	7-11	-150	0.9	-6.5	0.0	-1.0
P.b. – Rhiz. + *P.b.*	9-11	255	5.6	0.9	0.0	0.2

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

Table 14. Productivity and nutrient status of mature alfalfa harvested in 2005 (a single cut was harvested) at the Star City site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass ($kg\ ha^{-1}$)	tissue N[1] ($g\ kg^{-1}$)	N acquis.[2] ($kg\ ha^{-1}$)	tissue P[1] ($g\ kg^{-1}$)	P uptake ($kg\ ha^{-1}$)
Urea	1	2007	24.6	49.1	3.0	6.1
Ammon. nitrate	2	1938	24.7	48.2	3.1	5.8
Urea + Agro.	3	1948	24.7	47.5	2.9	5.4
ESN	4	1636	25.6	41.6	3.2	5.2
Ammon. sulphate	5	3928	28.7	112.9	2.7	10.7
Rhizobia (1X)	6	1655	23.9	39.5	2.9	4.8
Rhizobia (10X)	7	1566	25.2	39.5	3.0	4.6
TSP	8	2421	25.3	62.6	2.9	6.7
P. bilaiae	9	1788	24.3	43.5	3.0	5.4
P. bilaiae + ½ P	10	1767	22.8	42.2	2.8	5.1
Rhiz. + *P. bilaiae*	11	1843	24.2	43.0	3.0	5.2
MD	12	1468	25.6	37.3	3.3	4.7
MD + granule	13	1758	24.3	42.8	3.3	5.7
Undisturbed	14	1308	25.3	32.8	3.3	4.2
LSD (0.10)		*787*	*21.2*	*1.8*	*0.6*	*2.1*
Contrasts	*Trt compared*			***Difference between means***[1]		
Disturbed – undisturbed controls	14-12,13	610*	-0.6	14.4*	0.1	1.9*
No granule – granule	12-13	-290	1.2**	-5.4	0.0	-1.1*
N fertilizer – control	1,2,3,4-13	497	2.2	15.3	-1.0*	-0.5
N fertilizer – rhizobia	1,2,3,4-6,7	1087***	1.3	28.3***	0.4	3.4***
Rhizobia – control	6,7-13	-295	0.4	-6.5	-0.7**	-1.9*
P fertilizer – *P. bilaiae*	9-8	633	0.9*	19.1	-0.1	1.3
N fertilizer – slow release N fertilizer	1,2 -3,4	360	-0.9	8.1	-0.1	1.3
S fertilizer – control	5-12	2460***	3.2***	75.6***	-0.6***	6.1***
S – control	5,13-12	2750***	1.9*	81.1***	-0.6*	7.1***
Rhiz. – Rhiz. + *P.b.*	7-11	276.7*	-1.0*	3.5	0.0	0.5
P.b. – Rhiz. + *P.b.*	9-11	55	-0.5	-0.1	-0.1	-0.2

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

Table 15. Productivity and nutrient status of mature alfalfa harvested in 2006 (one cut was harvested) at the Star City site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass ($kg\ ha^{-1}$)	tissue N[1] ($g\ kg^{-1}$)	N acquis.[2] ($kg\ ha^{-1}$)	tissue P[1] ($g\ kg^{-1}$)	P uptake ($kg\ ha^{-1}$)
Urea	1	1063	157.4	163.4	23.3	24.3
Ammon. nitrate	2	1235	172.7	204.6	22.7	24.5
Urea + Agro.	3	1346	156.9	208.6	20.9	25.1
ESN	4	1239	164.2	197.1	23.7	26.6
Ammon. sulphate	5	2094	159.5	330.4	21.1	40.9
Rhizobia (1X)	6	1207	165.5	197.5	22.9	27.3
Rhizobia (10X)	7	1070	174.5	183.6	22.8	23.6
TSP	8	1620	166.6	262.8	22.2	33.2
P. bilaiae	9	658	174.2	113.6	28.9	18.3
P. bilaiae + ½ P	10	914	176.2	161.6	24.3	20.7
Rhiz. + *P. bilaiae*	11	1300	167.8	216.8	22.4	27.9
MD	12	1058	162.4	171.9	21.9	21.4
MD + granule	13	934	169.1	1556.0	27.8	23.9
Undisturbed	14	929	161.5	149.9	21.8	20.2
LSD (0.10)		*1027*	*154*	*22.0*	*6.9*	*16.4*
Contrasts	*Trt compared*	-------------------	***Difference between means[1]***		-------------------	
Disturbed – undisturbed controls	14-12,13	135	8.5	28.0	6.0*	4.8
No granule – granule	12-13	124	-6.7	15.9	-5.9***	-2.5
N fertilizer – control	1,2,3,4-13	1144	-25.1	149.7	-20.6***	5.0
N fertilizer – rhizobia	1,2,3,4-6,7	329	-28.8*	11.5	-0.9	-1.3
Rhizobia – control	6,7-13	408	1.8	69.1	-9.9***	3.1
P fertilizer – *P. bilaiae*	9-8	963***	-7.6	149.3***	-6.7***	15.0***
N fertilizer – slow release N fertilizer	1,2 -3,4	-288	9.1	-37.7	1.4	-2.8
S fertilizer – control	5-12	1035***	-2.9	158.6***	-0.8	19.6***
S – control	5,13-12	912*	3.7	142.7*	5.1	22.1***
Rhiz. – Rhiz. + *P.b.*	7-11	230	-6.8	33.2	-0.4	4.3
P.b. – Rhiz. + *P.b.*	9-11	642**	-6.4	103**	-6.5***	9.6

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

usually placed with the seed or directly below the seed. As the seed germinates the organism (Rhizobia or *P. bilaiae*) is in direct contact with the root system and hence in an ideal position to enhance P uptake.

Tissue S concentrations in unamended control plots were at deficient levels at Star City (Table 16). This was the only site where we examined the tissue S concentrations in the gypsum amended plants. In 2004 tissue S concentrations were very low. Both S amendments increased the concentrations to adequate levels of approximately 3.5 mg g^{-1}. The total amount of S taken up by the alfalfa in 2004 from the gypsum treatment plots was 7.6 kg ha^{-1} and was almost equivalent to the amount of S applied in the gypsum (7.4 kg S ha^{-1}). This explains the limited response to the gypsum in 2005 and the total lack of response in 2006.

Table 16. Mean S concentration and uptake (± standard error) in first cut alfalfa amended with ammonium sulphate fertilizer or gypsum in 2004 at the Star City site (n=6).

Treatment[1]	**S-conc**	**S-uptake**[2]
	mg g^{-1}	*kg ha^{-1}*
Ammon. Sulphate	3.5 (0.5) a	8.8 (1.4) a
Gypsum	3.6 (0.3) a	7.6 (0.7) a
Control	1.6 (0.2) b	1.8 (0.4) b
	-------------- *P-value*[3]	-------------
	0.002	0.001

[1] Refer to Table 2 for a complete description of treatments and application methods.
[2] S-uptake (kg ha^{-1})= S-conc (mg g^{-1}) x harvested biomass (g m^{-2}) x 10 000 m^2 ha^{-1} x 0.001 kg g^{-1}.
[3] Level of probability that means are not significantly different according to the two-tailed paired sample Student's *t*-test.

Productivity and Nutrient Acquisition – Crooked River:

Like the Star City site, the Crooked River site also responded to S fertilization, although the effect was only apparent in the early harvest in the application year (Table 17) and in the single harvest in year two (Table 19). Increases in biomass were much less at this site compared to Star City, with the S fertilizer increasing biomass by 30% in 2004 and 17% in 2005. The gypsum granules increased productivity by 15% and 20% in the same two years. There was no residual effect of the S amendments into the third year.

Unlike the other two sites, this site did not benefit from mechanically disturbing the soil. This site had generally higher clay contents, but also lower bulk densities than the other sites (Table 1). The lower bulk density in particular may indicate that this site is more adequately aerated than the other two sites and might not benefit from the soil mixing resulting from mechanical disturbance. This was also the only site that was negatively affected by mechanical disturbance on the stand counts as discussed earlier (Table 5). The negative effects on the physical stand may have outweighed any positive effects on nutrient availability. Most reports in the literature indicate that the damage done to the stand tends to negate any benefits from improved fertilizer placement (Leyshon, 1982; Simons et al., 1995). Hoe openers were used to band the fertilizers between rows in the first study, and a double-disc drill in the second study. Of the two the disc opener caused less damage.

Crooked River was the only site to exhibit a stimulation in biomass production and N acquisition from the rhizobial inoculants (Table 17) compared to the N fertilizers. However, the stimulation did not persist and by the second harvest (Table 18) the advantage was no longer apparent.

There were no responses to any of the treatments carried into the third year of the study at this site (Table 20).

Table 17. Productivity and nutrient status of mature alfalfa harvested mid-season in 2004 (first of two cuts) at the Crooked River site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	**Trt No.**	**Biomass (kg ha^{-1})**	**tissue N[1] (g kg^{-1})**	**N acquis.[2] (kg ha^{-1})**	**tissue P[1] (g kg^{-1})**	**P uptake (kg ha^{-1})**
Urea	1	2290	37.6	90.1	2.7	6.5
Ammon. nitrate	2	2407	38.7	90.0	2.7	6.2
Urea + Agro.	3	2117	37.2	81.7	2.5	5.5
ESN	4	2020	37.4	77.4	2.6	5.5
Ammon. sulphate	5	2455	37.3	92.4	2.5	6.1
Rhizobia (1X)	6	2503	36.3	84.9	2.6	6.0
Rhizobia (10X)	7	2505	37.1	88.8	2.7	6.5
TSP	8	2503	37.8	92.7	3.0	7.5
P. bilaiae	9	1927	37.5	77.7	2.7	5.5
P. bilaiae + ½ P	10	2615	38.4	78.5	2.7	5.5
Rhiz. + *P. bilaiae*	11	1990	38.2	98.8	2.8	7.1
MD	12	1920	32.5	60.8	2.5	4.7
MD + granule	13	2215	38.0	80.9	2.6	5.7
Undisturbed	14	2113	36.7	74.5	2.9	5.9
LSD (0.10)		*812*	*35.5*	*2.4*	*0.3*	*2.8*
Contrasts	*Trt compared*			***Difference between means[1]***		
Disturbed – undisturbed controls	14-12,13	-92	-3.2	-7.4	-0.7*	-1.4
No granule – granule	12-13	-295	-5.4***	-20.*1	-0.1	-1.0
N fertilizer – control	1,2,3,4-13	-27	-0.9	15.7	-0.0	1.0
N fertilizer – rhizobia	1,2,3,4-6,7	-1183*	4.2*	-8.1	0.0	-1.3
Rhizobia – control	6,7-13	578	-2.6	11.9	0.0	-1.2
P fertilizer – *P. bilaiae*	9-8	577**	0.2	15.0	0.3*	1.9*
N fertilizer – slow release N fertilizer	1,2 -3,4	560*	1.7*	20.9	0.2	1.8
S fertilizer – control	5-12	535**	4.7***	31.6**	0.0	1.4
S – control	5,13-12	830*	10.1***	51.8**	0.2	2.4
Rhiz. – Rhiz. + *P.b.*	7-11	-515*	1.1	9.0	0.1	0.6
P.b. – Rhiz. + *P.b.*	9-11	63	0.6	20.1	0.1	1.6

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

Table 18. Productivity and nutrient status of mature alfalfa harvested late-season in 2004 (second of two cuts) at the Crooked River site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Urea	1	2636	38.0	96.4	2.7	7.0
Ammon. nitrate	2	2280	37.5	84.4	2.9	6.6
Urea + Agro.	3	2438	37.8	88.5	2.7	6.4
ESN	4	2527	34.1	94.8	2.7	7.4
Ammon. sulphate	5	2481	38.0	99.8	2.6	6.9
Rhizobia (1X)	6	2367	35.0	80.8	2.6	6.0
Rhizobia (10X)	7	2445	35.8	88.7	2.8	6.8
TSP	8	2530	37.3	94.2	3.0	7.6
P. bilaiae	9	2598	35.3	93.0	2.5	6.7
P. bilaiae + ½ P	10	2623	35.6	86.2	2.6	6.4
Rhiz. + *P. bilaiae*	11	2468	35.4	94.0	2.8	7.4
MD	12	2445	34.8	85.9	2.7	6.7
MD + granule	13	2430	35.6	85.9	2.7	6.4
Undisturbed	14	2460	33.5	78.6	2.6	6.0
LSD (0.10)		*602*	*24.6*	*6.3*	*0.4*	*1.9*
Contrasts	*Trt compared*	*Difference between means*[1]				
Disturbed – undisturbed controls	14-12,13	-45	3.3	14.6	0.2	1.1
No granule – granule	12-13	15	-0.8	0.1	0.1	0.3
N fertilizer – control	1,2,3,4-13	162	4.8	20.7	0.4	1.9
N fertilizer – rhizobia	1,2,3,4-6,7	258	5.7	25.1	0.2	1.7
Rhizobia – control	6,7-13	-48	-0.4	-2.2	0.1	0.1
P fertilizer – *P. bilaiae*	9-8	-68	2.1	1.1	0.5***	0.9
N fertilizer – slow release N fertilizer	1,2 -3,4	-48	3.6	-2.5	0.3	-0.2
S fertilizer – control	5-12	37	3.3*	13.8*	-0.1	0.2
S – control	5,13-12	22	4.1	13.7	-0.1	-0.1
Rhiz. – Rhiz. + *P.b.*	7-11	23	-0.4	5.3	0.0	0.6
P.b. – Rhiz. + *P.b.*	9-11	-130	0.1	0.9	0.3*	0.7

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

Table 19. Productivity and nutrient status of mature alfalfa harvested in 2005 (a single cut was harvested) at the Crooked River site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Urea	1	3360	35.2	120.3	2.9	10.0
Ammon. nitrate	2	3764	34.3	141.7	2.8	11.7
Urea + Agro.	3	3392	34.1	131.9	2.6	10.2
ESN	4	3152	34.4	122.6	2.9	10.3
Ammon. sulphate	5	3452	34.3	128.1	2.6	9.9
Rhizobia (1X)	6	3193	35.4	124.5	2.9	10.1
Rhizobia (10X)	7	3525	34.7	123.8	2.9	10. 2
TSP	8	3192	36.3	124.7	3.1	10.5
P. bilaiae	9	2835	36.7	111.9	3.0	9.1
P. bilaiae + ½ P	10	3700	34.2	126.2	2.8	10.4
Rhiz. + *P. bilaiae*	11	3358	34.6	128.5	2.9	11.0
MD	12	2953	36.0	121.5	2.8	9.5
MD + granule	13	3535	34.0	120.2	2.8	10.0
Undisturbed	14	3315	34.2	122.7	2.8	9.2
LSD (0.10)		*1037*	*35.5*	*3.0*	*0.3*	*3.1*
Contrasts	*Trt compared*	-------------------	-------------------	***Difference between means***[1]	-------------------	-------------------
Disturbed – undisturbed controls	14-12,13	-142	1.7	-3.6	0.1	-0.4
No granule – granule	12-13	-582*	2.0	1.3*	0	-0.5
N fertilizer – control	1,2,3,4-13	-473	1.9	35.7	-0.1	2.2
N fertilizer – rhizobia	1,2,3,4-6,7	230	-2.2	20.0	-0.3	1.4
Rhizobia – control	6,7-13	-352	2.0	7.9	0.1	0.4
P fertilizer – *P. bilaiae*	9-8	357	-0.3	12.8	0.1	1.5
N fertilizer – slow release N fertilizer	1,2 -3,4	580	1.0	7.5	0.3*	1.2
S fertilizer – control	5-12	498*	-1.6	6.6*	-0.2*	0.4
S – control	5,13-12	1080*	-3.6	5.4*	-0.2	0.8
Rhiz. – Rhiz. + *P.b.*	7-11	-167	-0.1	4.7	0.1	0.7
P.b. – Rhiz. + *P.b.*	9-11	63	0.6	20.1	0.1	1.6

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

Table 20. Productivity and nutrient status of mature alfalfa harvested in 2006 (a single cut was harvested) at the Crooked River site. Treatments were applied to mature stands in the spring of 2004. Details of the treatments are outlined in Table 2.

Treatment	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Urea	1	5143	188.4	1004	1.7	8.9
Ammon. nitrate	2	5628	173.4	977	1.6	9.0
Urea + Agro.	3	5285	181.3	851	1.6	8.8
ESN	4	4883	180.6	882	1.7	8.1
Ammon. sulphate	5	4662	180.2	837	1.8	8.1
Rhizobia (1X)	6	5273	182.5	963	1.6	8.6
Rhizobia (10X)	7	5117	179.4	1042	1.7	9.6
TSP	8	5302	199.4	1039	1.7	8.8
P. bilaiae	9	5277	179.7	954	1.6	8.6
P. bilaiae + ½ P	10	4810	177.7	923	1.7	8.9
Rhiz. + *P. bilaiae*	11	4834	187.9	813	1.7	8.0
MD	12	5114	150.6	776	1.7	8.3
MD + granule	13	5160	182.0	942	1.8	9.1
Undisturbed	14	5495	174.6	965*	1.6	8.9
LSD (0.10)		*1945*	*39.3*	*38.0*	*0.3*	*3.5*
Contrasts	*Trt compared*	- - - - - - - -	- - - - - - - -	***Difference between means***[1]	- - - - - - - -	- - - - - - - -
Disturbed – undisturbed controls	14-12,13	-715	-17.7	-212	1.7	-0.5
No granule – granule	12-13	-45	-31.4	-166	-0.9	-0.8
N fertilizer – control	1,2,3,4-13	302	-4.3	116	-4.3	-1.4
N fertilizer – rhizobia	1,2,3,4-6,7	161	-0.1	-127	-0.2	-1.7
Rhizobia – control	6,7-13	70.2	-2.1	122	-2.0	0.1
P fertilizer – *P. bilaiae*	9-8	25.5	19.7***	84	0.4	0.2
N fertilizer – slow release N fertilizer	1,2 -3,4	602	-0.2	77	0.8	0.9
S fertilizer – control	5-12	-453	29.6	61	1.1*	-0.2
S – control	5,13-12	-408	61.1	227	2.0	0.6
Rhiz. – Rhiz. + *P.b.*	7-11	-281	8.4	-228	0	-1.6
P.b. – Rhiz. + *P.b.*	9-11	-442	8.2	-14.1	0.4	-0.6

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biobgical N fixation.

Like alfalfa at the Smeaton site, tissue S concentrations were adequate although on the low side. Applying S fertilizer to the alfalfa did not increase S concentrations significantly ($p > 0.10$) by the first cut of the application year (Table 21). However, there was a trend toward higher tissue S concentrations in the fertilized plants. It seems likely that tissue concentrations may have been higher later in the year or in the following year as the S fertilizer was translocated into the rooting zone. Clearly though, response to S fertilization was limited at this site indicating that the site was not severely deficient in S.

Soils tests for the three sites (Table 1) confirm that the Star City site was the most deficient in plant available S.

Table 21. Mean S concentration and uptake (± standard error) in alfalfa amended with ammonium sulphate fertilizer as well as a control for the first cut in 2004 at the Crooked River site (n=6).

Treatment[1]	S-conc	S-uptake[2]
	mg g^{-1}	*kg ha^{-1}*
21-0-0-24	3.4 (0.3)	8.8 (1.4)
Control	2.7 (0.3)	5.8 (0.9)
	------------ *P-value*[3] ------------	
	0.26	0.15

[1]Refer to Table 2 for a complete description of treatments and application methods.
[2] S-uptake (kg ha^{-1})= S-conc (mg g^{-1}) x harvested biomass (g m^{-2}) x 10 000 m^{2} ha^{-1} x 0.001 kg g^{-1}.
[3] Level of probability that means are not significantly different according to the two-tailed paired sample Student's *t*-test.

Sinorhizobium meliloti populations:

S. meliloti population numbers in the soil were high at all three sites prior to inoculation (Table 22), indicating that there were existing populations of rhizobium that were capable of nodulating alfalfa. Application of the rhizobial inoculant at 10 X the seeding application rate had a tendency to increase the numbers of bacteria counted in the MPN assay. However, the numbers were not increased to levels where they affect N-fixation significantly. The Smeaton site exhibited the greatest increase in *S. meliloti* populations by the application of the inoculant, but was completely unresponsive in terms of biomass production or N acquisition. Clearly the existing populations of *S. meliloti* in the soil continue to actively fix N through the lifespan of the alfalfa stand.

Table 22. Mean *S. meliloti* population ratings (± standard error) of soils from three sites sampled in June 2005 (n=3).

Treatment[1]		**Smeaton**	**Star City**	**Crooked River**
		----------------	*Rating*[2]	----------------
4-kg ha^{-1} *S. meliloti*		4.0 (0.6)	4.7 (0.3)	4.7 (0.3)
40-kg ha^{-1} *S. meliloti*		5.0 (0.0)	5.0 (0.0)	5.0 (0.0)
Control		4.0 (0.6)	4.7 (0.3)	4.7 (0.3)
		ANOVA		
Source of Variation	*df*	----------------	*P-value*[3]	----------------
Treatment	2	0.69	0.69	0.25

[1] Refer to Table 2 for a complete description of treatments and application methods.
[2] Soil samples rated from 1-5 by number *S. meliloti* cells present (rhizobia g^{-1}). [3]Probability level of analysis of variance.

Delayed Inoculation Study:

The delayed inoculation study examined the effectiveness of inoculating alfalfa with *S. meliloti* the year after the alfalfa was seeded. Rhizobium applications as well as applications of *P. bilaiae* were compared to fertilizer applications. Controls (inoculants and fertilizer) were established in 2004 and then the delayed treatments were applied in 2005. Measurements were made in 2005 and 2006.

The most common practice for producers growing alfalfa is to sow preinoculated seed purchased from the seed distributor. However, many producers purchase and sow seed without knowing if it is inoculated at all. Seed left over from the previous year can be seeded, but will have lost the viability of the applied rhizobium with storage. There is also a common perception that if a field was inoculated in the past, the rhizobia will remain in the soil almost indefinitely and that yearly inoculation is not necessary. There are reports in the literature for both forage legumes and annual pulses, where inoculation was ineffective compared to uninoculated controls.

In this study, at both sites inoculation with *S. meliloti* was effective in increasing biomass production and N fixation in the alfalfa (Tables 23 & 26). For biomass production, the largest increase between inoculated plants and controls occurred when the inoculant was applied at the time of seeding. The Arborfield site was particularly responsive to the applied rhizobia (Table 23). However, by the second season early harvest (Tables 24 & 27) the delayed inoculation treatment also exhibited higher yields compared to the disturbed control. Nitrogen derived from fixation (Ndfa) was consistently higher in the *Rhizobium* inoculated plants compared to all others. More importantly there was very little difference in the productivity, N content, N acquisition and N fixation between those plots inoculated with *S. meliloti*, at seeding or delayed by one year in 2005 or 2006. Delaying the application of the rhizobial inoculant was as effective as application at seeding.

Alfalfa inoculated with the rhizobial inoculants maintained higher percentages of N derived from fixation than the untreated controls into the second harvest of the second season (Tables 25 & 28).

According to the contrast analyses performed for the Arborfield site, the treatments applied in 2004 were more productive than the uninoculated controls established in 2004, and all of the treatments applied in 2005 were more productive than the controls established in 2005

(Tables 23-25), with the exception of the 2005 treatments harvested late in 2006. As the stands aged fewer treatment responses were evident. It would appear that as the plants establish and the root systems expand the stands become more and more homogeneous.

Biomass production at the Aberdeen site was less responsive than the Arborfield site, but application of the treatments did improve productivity compared to the control plots in the first harvest (Table 26). The control treatment that was established in 2005 was somewhat of an anomaly. In 2006, at both harvests, this uninoculated control treatment was the highest producing treatment, and also had the highest N and P uptake (Table 28). We have no explanation for this observation.

Treatments targeted at P fertility were all generally similar at the Arborfield site (Tables 23-25). In general, P fertilization and inoculation with *P. bilaiae* tended to increase biomass production regardless of the year they were applied. However, the *P. bilaiae* plus ½ P fertilizer treatment did not perform well in 2005 (Table 23) but these plots had reached the same level of productivity by 2006 (Tables 24 & 25). *Penicillium bilaiae* applied alone was superior when applied the year after sowing (2005) compared to the year of sowing (2004). It may be that the placement of the granule the year after sowing resulted in better contact with the root system. Arborfield was moderately responsive to P placement in general; alfalfa grown in the rhizobia treatments (without additional P) and the 2005 control, all exhibited lower P uptake compared to the other treatments.

The Aberdeen site was generally more variable in its responses to P applications than the Arborfield site (Tables 26-28). Those treatments that included *P. bilaiae* all had the highest P uptake, compared to other treatments in the first year of the study (Table 26). Also at this site, application of N fertilizer increased P uptake compared to controls. As was the case at the Arborfield site, the year of application had little effect on the performance of the P fertilizers and *P. bilaiae*.

Table 23. Productivity and nutrient status of alfalfa sampled in 2005 at Arborfield, SK. Treatments were fertilized or inoculated one year after the alfalfa was seeded compared to year of seeding treatments. Year of seeding treatments were applied in 2004; delayed treatments were applied in 2005.

Treatment	Treat. Year	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	Ndfa[3] (%)	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Control	2004	1	4725	24.9	115.2	78	2.7	12.6
N-fertilizer	2004	2	5200	19.5	100.0	80	2.4	12.4
P-fertilizer	2004	3	4913	19.0	92.6	81	2.4	11.6
N & P fertilizer	2004	4	4428	23.0	102.1	80	2.7	11.8
Rhizobia	2004	5	5470	17.2	95.0	84	2.0	10.9
P. bilaiae	2004	6	5183	19.4	101.8	81	2.4	12.2
Rhizobia & *P. bilaiae*	2004	7	5040	19.7	100.0	83	2.4	11.9
P. bilaiae + ½ P	2004	8	4832	19.3	96.7	81	2.3	11.4
Rhizobia	2005	9	4723	19.9	93.2	84	2.3	10.5
P. bilaiae	2005	10	5473	21.4	115.8	81	2.5	13.6
Rhizobia & *P. bilaiae*	2005	11	5057	23.4	122.3	79	2.7	13.8
P. bilaiae + ½ P	2005	12	4760	21.1	107.4	79	2.5	12.3
Control	2005	13	4675	20.0	91.6	78	2.2	10.2
LSD (0.10)			*1217*	*5.2*	*38.9*	*7.2*	*0.4*	*3.5*
				------------------ *Difference between means*[1]	------------------			
Contrasts		*Trt compared*						
control – treatments (2004)		1-2,3,4,5,6,7	-19992***	37.1***	118.6	-3.4	2.4*	5.9
control - treatment (2005)		13 - 9,10,11,12	-1313**	-5.9	-72.4	-2.8	-1.1	-9.3**
2004-2005		5,6,7,8-9,10,11,12	512	-10.2**	-45.4	-1.6	-0.9*	-3.8
N fertilizer – Rhizobia		2-5,9	207	1.8	11.6	-4.0**	0.6**	3.4**
P fertilier – *P. bilaiae*		3-6,10,8,12	595	-5.3	-51.4	-0.5	0.0	-3.1
N & P fertilizer – Rhiz. + *P. bilaiae*		4-7,11	1240	2.8	-18.1	-1.0	0.2	-.2.
Rhizobia 2004-Rhizobia 2005		5-9	747	-2.7	1.6	0	-0.3	0.4
P. bilaiae 2004 – *P. bilaiae* 2005		6-10	290	-1.9	-14.0	0	-0.2	-1.4*
Rhiz. + P. bil. 2004 - Rhiz. + P. bil. 2005		7-11	17	-3.7*	-22.3	4.0	-0.3*	-1.9
Rhiz. + ½ P 2004 - Rhiz. + ½ P 2005		8-12	72	-1.8	-10.8	2.0	-0.2	-0.9

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

[3] Nitrogen derived from atmosphere measured by isotope dilution method

Table 24. Productivity and nutrient status of alfalfa sampled early season (first cut of two) in 2006 at Arborfield, SK. Treatments were fertilized or inoculated one year after the alfalfa was seeded compared to year of seeding treatments. Year of seeding treatments were applied in 2004; delayed treatments were applied in 2005.

Treatment	Treat. Year	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	Ndfa[3]	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Control	2004	1	5991	24.3	147.5	76	2.4	14.5
N-fertilizer	2004	2	6336	22.3	142.8	77	2.3	14.6
P-fertilizer	2004	3	5950	22.2	130.7	76	2.2	12.8
N & P fertilizer	2004	4	6255	26.2	164.8	75	2.6	15.9
Rhizobia	2004	5	5935	21.5	127.9	80	2.3	13.3
P. bilaiae	2004	6	6382	23.4	150.7	76	2.3	14.9
Rhizobia & *P. bilaiae*	2004	7	5778	23.4	139.2	79	2.3	13.5
P. bilaiae + ½ P	2004	8	6648	24.4	159.9	80	2.4	16.1
Rhizobia	2005	9	6162	24.2	149.6	81	2.3	14.3
P. bilaiae	2005	10	6730	21.7	118.3	77	2.2	11.8
Rhizobia & *P. bilaiae*	2005	11	6579	22.89	151.3	80	2.2	14.8
P. bilaiae + ½ P	2005	12	6552	22.9	150.8	78	2.3	14.8
Control	2005	13	5852	22.8	137.2	77	2.4	14.0
LSD (0.10)			*1537*	*3.6*	*46.4*	*5.3*	*0.3*	*4.1*
Contrasts		*Trt compared*			***Difference between means***[1]			
control – treatments (2004)		1-2,3,4,5,6,7	-1348**	6.7	16.3	-1.2	0.5	0.5
control - treatment (2005)		13 - 9,10,11,12	-1613**	-0.4	-21.3	-2.0	0.5*	0.3
2004-2005		5,6,7,8-9,10,11,12	-279	0.9	7.8	-0.2	0.3	2.0
N fertilizer – Rhizobia		2-5,9	547	-1.2	8.0	3.5	0.4	1.6
P fertilicr – *P. bilaiae*		3-6,10,8,12	-1515**	-3.8	-57.0	-1.8	-0.6	-6.3
N & P fertilizer – Rhiz. + *P. bilaiae*		4-7,11	153	6.1**	38.8	-4.5	0.6***	3.6
Rhizobia 2004-Rhizobia 2005		5-9	-227	-2.7*	-21.7	-1.0	0.0	-1.0
P. bilaiae 2004 – *P. bilaiae* 2005		6-10	652	1.6	32.5*	-1.0	0.1	3.0*
Rhiz. + P. bil. 2004 - Rhiz. + P. bil. 2005		7-11	-800	0.5	-12.1	-1.0	0.1	-1.4
Rhiz. + ½ P 2004 - Rhiz. + ½ P 2005		8-12	97	1.5	-9.2	-2.0	0.2	1.4

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

[3] Nitrogen derived from atmosphere measued by isotope dilution method

Table 25. Productivity and nutrient status of alfalfa sampled late season (second cut of two) in 2006 at Arborfield, SK. Treatments were fertilized or inoculated one year after the alfalfa was seeded compared to year of seeding treatments. Year of seeding treatments were applied in 2004; delayed treatments were applied in 2005.

Treatment	Treat. Year	Trt No.	Biomass ($kg\ ha^{-1}$)	tissue N[1] ($g\ kg^{-1}$)	N acquis.[2] ($kg\ ha^{-1}$)	Ndfa[3] (%)	tissue P[1] ($g\ kg^{-1}$)	P uptake ($kg\ ha^{-1}$)
Control	2004	1	3691	31.9	116.9	73	2.3	8.6
N-fertilizer	2004	2	3516	30.9	107.4	71	2.4	8.2
P-fertilizer	2004	3	3685	31.2	114.7	72	2.3	8.3
N & P fertilizer	2004	4	3420	32.8	110.2	71	2.4	7.9
Rhizobia	2004	5	3288	32.0	106.3	79	2.4	8.0
P. bilaiae	2004	6	3498	32.3	114.7	75	2.4	8.5
Rhizobia & *P. bilaiae*	2004	7	3408	30.3	102.5	79	2.3	7.8
P. bilaiae + ½ P	2004	8	3696	30.9	113.6	77	2.3	8.5
Rhizobia	2005	9	3548	30.6	108.2	81	2.2	8.0
P. bilaiae	2005	10	3415	31.6	107.3	74	2.4	8.1
Rhizobia & *P. bilaiae*	2005	11	3498	31.7	110.0	78	2.3	8.0
P. bilaiae + ½ P	2005	12	3284	31.0	101.7	71	2.3	7.6
Control	2005	13	3526	32.2	113.3	71	2.4	8.3
LSD (0.10)			*585*	*2.9*	*16.1*	*6.1*	*0.2*	*1.4*
Contrasts		*Trt compared*	-------------------	***Difference between means***[1]	-------------------			
control – treatments (2004)		1-2,3,4,5,6,7	1325	2.9	49.0	-1.5	0.0	3.1
control - treatment (2005)		13 - 9,10,11,12	358	4.2	26.3	-5.0	0.3	1.8
2004-2005		5,6,7,8-9,10,11,12	146	0.7	10.2	1.5	0.1	1.1
N fertilizer – Rhizobia		2-5,9	196	-0.7	0.2	-9.0**	0.0	0.4
P fertilier – *P. bilaiae*		3-6,10,8,12	849	-0.9	21.5	-2.3	-0.3	0.7
N & P fertilizer – Rhiz. + *P. bilaiae*		4-7,11	-66	3.6*	8.0	-7.5**	0.1	0.0
Rhizobia 2004-Rhizobia 2005		5-9	-259	1.4	-2.0	-2.0	0.1*	0.0
P. bilaiae 2004 – *P. bilaiae* 2005		6-10	83	0.7	7.5	1.0	0.0	0.4
Rhiz. + P. bil. 2004 - Rhiz. + P. bil. 2005		7-11	89	-1.4	-7.2	-1.0	0.0	-0.2
Rhiz. + ½ P 2004 - Rhiz. + ½ P 2005		8-12	412*	-0.1	11.9*	6.0**	0.0	0.9

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

[3] Nitrogen derived from atmosphere measued by isotope dilution method

Table 26. Productivity and nutrient status of alfalfa sampled in 2005 at Aberdeen, SK. Treatments were fertilized or inoculated one year after the alfalfa was seeded compared to year of seeding treatments. Year of seeding treatments were applied in 2004; delayed treatments were applied in 2005.

Treatment	Treat. Year	Trt No.	Biomass ($kg\ ha^{-1}$)	tissue N[1] ($g\ kg^{-1}$)	N acquis.[2] ($kg\ ha^{-1}$)	Ndfa[3] (%)	tissue P[1] ($g\ kg^{-1}$)	P uptake ($kg\ ha^{-1}$)
Control	2004	1	5367	15.0	80.3	84	1.4	7.7
N-fertilizer	2004	2	5998	17.3	105.7	80	1.8	10.9
P-fertilizer	2004	3	5798	16.0	92.2	78	1.6	9.4
N & P fertilizer	2004	4	5643	16.5	95.6	80	1.7	9.8
Rhizobia	2004	5	5578	16.0	89.2	82	1.5	8.3
P. bilaiae	2004	6	6277	18.6	114.6	80	1.8	11.3
Rhizobia & *P. bilaiae*	2004	7	5197	18.1	93.2	80	1.7	8.9
P. bilaiae + ½ P	2004	8	5067	16.7	99.7	76	1.8	10.5
Rhizobia	2005	9	5613	18.1	101.9	81	1.7	9.7
P. bilaiae	2005	10	6255	16.7	103.0	79	1.7	10.3
Rhizobia & *P. bilaiae*	2005	11	5840	14.7	88.1	79	1.5	9.0
P. bilaiae + ½ P	2005	12	5552	14.8	80.1	77	1.5	8.2
Control	2005	13	5648	19.3	103.9	72	1.6	8.7
LSD (0.10)			*1284*	*5.8*	*36.6*	*5.2*	*0.5*	*3.6*
Contrasts		*Trt compared*	-------	-------	***Difference between means[3]***	-------	-------	
control – treatments (2004)		1-2,3,4,5,6,7	-2991	-14.4	-128.5	-16***	-1.8	-14.9*
control - treatment (2005)		13 - 9,10,11,12	-667	13.0*	42.6	-7**	-0.2	-2.4
2004-2005		5,6,7,8-9,10,11,12	-142	5.2	23.6	0.5	0.3	1.8
N fertilizer – Rhizobia		2-5,9	805	0.6	20.4	-1.5	0.3	3.9
P fertilier – *P. bilaiae*		3-6,10,8,12	-958	-2.8	-28.7	0	-0.4	-2.9
N & P fertilizer – Rhiz. + *P. bilaiae*		4-7,11	250	0.2	9.7	-0.5	0.1	1.8
Rhizobia 2004-Rhizobia 2005		5-9	-35	-2.0	-12.6	1.0	-0.3	-1.4
P. bilaiae 2004 – *P. bilaiae* 2005		6-10	22	1.9	11.6	1.0	0.1	1.0
Rhiz. + P. bil. 2004 - Rhiz. + P. bil. 2005		7-11	-643	3.4	5.0	1.0	0.2	-0.1
Rhiz. + ½ P 2004 - Rhiz. + ½ P 2005		8-12	515	1.9	19.6	-1.0	0.3	2.2

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

[3] Nitrogen derived from atmosphere measued by isotope dilution method

Table 27. Productivity and nutrient status of alfalfa sampled early season (first cut of two) in 2006 at Aberdeen, SK. Treatments were fertilized or inoculated one year after the alfalfa was seeded compared to year of seeding treatments. Year of seeding treatments were applied in 2004; delayed treatments were applied in 2005.

Treatment	Treat. Year	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	Ndfa[3] (%)	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Control	2004	1	5982	25.7	155.2	70	1.3	7.7
N-fertilizer	2004	2	5968	28.3	167.2	80	1.4	8.5
P-fertilizer	2004	3	6480	25.1	163.4	78	1.3	8.4
N & P fertilizer	2004	4	5705	25.7	146.3	81	1.3	7.4
Rhizobia	2004	5	6796	25.7	177.3	82	1.3	9.1
P. bilaiae	2004	6	6436	24.5	158.5	79	1.2	7.8
Rhizobia & *P. bilaiae*	2004	7	5273	24.1	128.2	78	1.2	6.4
P. bilaiae + ½ P	2004	8	5701	22.9	131.3	80	1.2	7.1
Rhizobia	2005	9	6295	24.8	157.6	83	1.3	8.1
P. bilaiae	2005	10	6107	25.7	157.4	76	1.3	8.0
Rhizobia & *P. bilaiae*	2005	11	5456	25.9	140.4	82	1.3	7.1
P. bilaiae + ½ P	2005	12	5387	24.5	132.7	75	1.2	6.6
Control	2005	13	6144	25.9	159.3	69	1.3	8.0
LSD (0.10)			1250	3.5	40.3	5.4	0.3	2.7
Contrasts		*Trt compared*	-------------------	-------------------	***Difference between means***[1]	-------------------	-------------------	
control – treatments (2004)		1-2,3,4,5,6,7	-490	3.9	14.6	-9.7***	-0.3	-0.9
control - treatment (2005)		13 - 9,10,11,12	1331	2.6	49.1	-10.0***	0.3	2.0
2004-2005		5,6,7,8-9,10,11,12	964	-3.9	7.0	0.8	-1.5	0.5
N fertilizer – Rhizobia		2-5,9	-1155	6.1**	-0.5	-2.5	3.1	-0.1
P fertilizer – *P. bilaiae*		3-6,10,8,12	2288	2.8	73.9	1.5	1.9	4.0
N & P fertilizer – Rhiz. + *P. bilaiae*		4-7,11	681	1.4	24.1	1.0	1.1	1.4
Rhizobia 2004-Rhizobia 2005		5-9	501	0.8	9.70	-1.0	0.6	0.9
P. bilaiae 2004 – *P. bilaiae* 2005		6-10	329	-1.3	1.1	3.0	-1.2	-0.3
Rhiz. + P. bil. 2004 - Rhiz. + P. bil. 2005		7-11	-182	-1.8	-12.3	4.0*	-0.9	-0.6
Rhiz. + ½ P 2004 - Rhiz. + ½ P 2005		8-12	315	-1.7	-1.4	5.0**	0.1	0.5

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

[3] Nitrogen derived from atmosphere measued by isotope dilution method

Table 28. Productivity and nutrient status of alfalfa sampled late season (second cut of two) in 2006 at Aberdeen, SK. Treatments were fertilized or inoculated one year after the alfalfa was seeded compared to year of seeding treatments. Year of seeding treatments were applied in 2004; delayed treatments were applied in 2005.

Treatment	Treat. Year	Trt No.	Biomass (kg ha^{-1})	tissue N[1] (g kg^{-1})	N acquis.[2] (kg ha^{-1})	Ndfa[3] (%)	tissue P[1] (g kg^{-1})	P uptake (kg ha^{-1})
Control	2004	1	3684	22.6	80.0	67	8.6	30.4
N-fertilizer	2004	2	3318	23.1	75.9	78	8.8	29.6
P-fertilizer	2004	3	3770	23.2	87.7	76	9.3	35.0
N & P fertilizer	2004	4	3440	24.6	84.1	78	9.8	33.7
Rhizobia	2004	5	3679	23.1	84.2	80	9.1	33.5
P. bilaiae	2004	6	3658	22.6	82.2	77	8.8	32.6
Rhizobia & *P. bilaiae*	2004	7	3788	24.6	94.0	79	10.0	38.3
P. bilaiae + ½ P	2004	8	3665	23.9	87.9	75	9.8	36.3
Rhizobia	2005	9	3572	24.1	85.9	79	9.3	33.3
P. bilaiae	2005	10	4049	21.3	86.1	77	8.8	35.4
Rhizobia & *P. bilaiae*	2005	11	3660	23.6	83.9	79	9.7	35.4
P. bilaiae + ½ P	2005	12	2754	25.5	68.0	71	10.3	27.2
Control	2005	13	4375	21.9	95.8	70	8.8	38.7
LSD (0.10)			807	3.3	18.0	4.7	1.6	9.2
Contrasts		*Trt compared*	---------	---------	*Difference between means*[1]	---------	---------	---------
control – treatments (2004)		1-2,3,4,5,6,7	472	-6.9	-36.9	-11***	-5.2	-26.1
control - treatment (2005)		13 - 9,10,11,12	3466***	-6.9**	59.3***	-6.5**	-2.8	23.5*
2004-2005		5,6,7,8-9,10,11,12	755	-0.4	24.3	1.3	-0.4	9.3
N fertilizer – Rhizobia		2-5,9	-615	-1.0	-18.4	-1.5	-0.7	-7.5
P fertilier – *P. bilaiae*		3-6,10,8,12	953	-0.4	26.6	1.0	-0.5	8.6
N & P fertilizer – Rhiz. + *P. bilaiae*		4-7,11	-568	1.1	-9.7	-1.0	0.0	-6.3
Rhizobia 2004-Rhizobia 2005		5-9	107	-1.00	-1.6	1.0	-0.2	0.2
P. bilaiae 2004 – *P. bilaiae* 2005		6-10	-391	1.2	-4.0	0	0.1	-2.8
Rhiz. + P. bil. 2004 - Rhiz. + P. bil. 2005		7-11	128	1.0	10.0	0	0.2	2.9
Rhiz. + ½ P 2004 - Rhiz. + ½ P 2005		8-12	911***	-1.6	19.9***	4.0*	-0.5	9.1**

*, **, *** indicate differences are significant at the 0.2, 0.1, and 0.05 levels of significance.

[1] grams of nutrient per kg of above ground biomass

[2] N acquisition is the total N in the above-ground biomass per hectare of land and includes both N taken up from the soil and biological N fixation.

[3] Nitrogen derived from atmosphere measued by isotope dilution method

e) Conclusions and Recommendations:

Mechanical disturbance from the disc-banding operation used in this study caused little disturbance to the stands and did not affect the stand densities to the point of reducing yields. At the two sites that had compacted soils the mechanical disturbance actually stimulated N uptake and slightly increased yields.

None of the treatments aimed at improving P fertility affected nutrient uptake nor yield in the mature alfalfa stands, even though soil tests indicated that P was potentially limiting. Alfalfa appears to be well adapted to extracting P from the soil.

Applications of N fertilizers increased N uptake and to a lesser extent yield in the year that they were applied. Readily available N products (urea and ammonium nitrate) were more effective than the slow release products. The slow release fertilizers did not appear to become available over time to the extent that they affected productivity in the stand. Despite the response to N fertilizers, the impact was short lived and only affected nutrition and yield in the year of application. Fertilization with N would need to be followed yearly, which is not practical nor economic for alfalfa producers.

Star City was the site with the poorest fertility, and responded most strongly to the largest number of treatments. While this site did respond with improved N fertility to reinoculation with rhizobia, the response was small. Star City was severely S deficient and responded strongly to S fertilization. Although we broadcasted ammonium sulphate on the surface of the soil, there are indications that soil placement (banding) may have been beneficial. Sulphur in the gypsum granules (the carrier for the microbial inoculants) banded into the soil was more quickly available for plant uptake than was the S fertilizer. The fertilizer required precipitation and time for the S to be carried into the rooting zone. All of the sites showed tendencies for some degree of response to the S applications. It appears that S deficiency in alfalfa stands may be more widespread than is currently recognized.

Delaying application of rhizobial and *P. bilaiae* inoculants by a year in newly seeded crops, had no effect on yield, N fixation and overall plant nutrition. In a situation where a newly seeded stand did not nodulate properly, re-applying an inoculant early the following year is a viable option. Unlike the mature alfalfa stands, the new stands responded to the application of P fertilizer and especially *P. bilaiae*. This is probably

because the root systems of the new stands are not developed enough to maximally extract P from the soil.

f) Acknowledgements: Saskatchewan Agriculture and Food has been acknowledged as the funding agency supporting this work at all of conferences, workshops, field days and tours that this work has been presented at.

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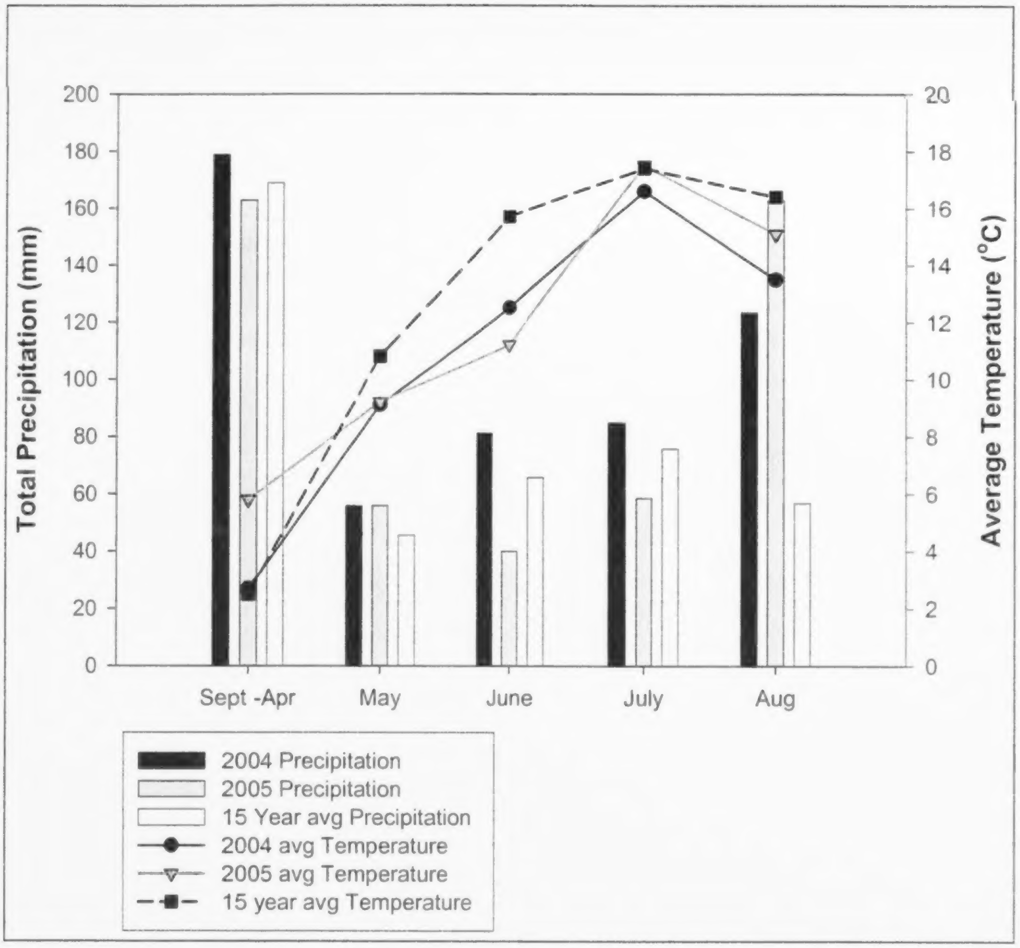
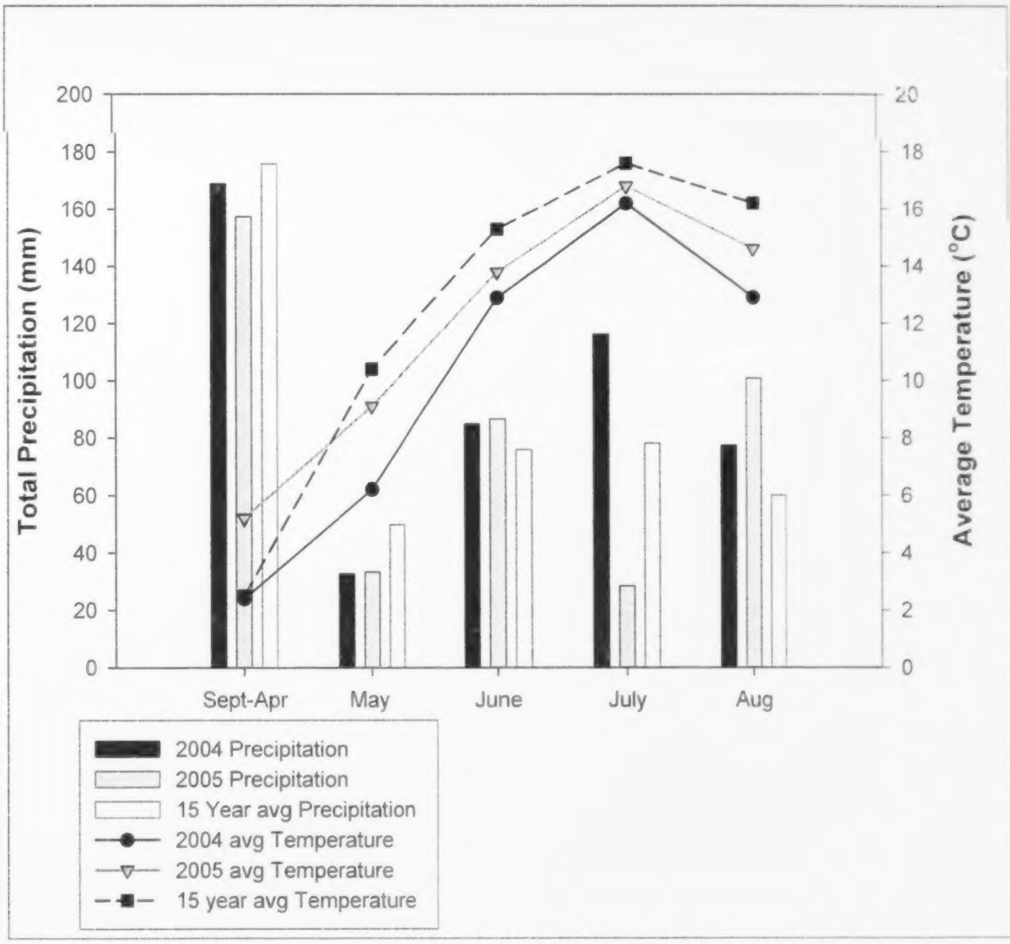
Appendices



Appendix 1. Annual precipitation and growing season average monthly air temperature for the Star City and Crooked River sites (as recorded from the Tisdale Environment Canada weather station).



Appendix 2. Annual precipitation and growing season average monthly air temperature for the Smeaton site (as recorded from the Nipawin Environment Canada weather station).

h) Administrative:

Outputs from project:

J.D. Knight. 2007. Evaluation of inoculant delivery formulations for alfalfa yield and nitrogen fixation. accepted for publication in Canadian Journal of Plant Science. *In Press.*

K. Farden, and J.D. Knight. 2006. Strategies for Improving Soil Fertility in Mature Alfalfa Stands. Canadian Society of Soil Science Annual Meeting. May 14-17. Banff. Alberta
K. Farden and J.D. Knight. Use of inoculants to improve fertility in forage stands. Forage Technical Updates 2006. June 5, 2006, Swift Current, SK (20 ppl)

J.D. Knight. Managing soil fertility organically. Organic Update February 15th, 2006, Bruno, SK (150 ppl).

J.D. Knight. Use of inoculants to improve fertility in forage stands. Forage Technical Updates 2006. June 07, 2006, Weyburn, SK (30 ppl)

J.D. Knight. Use of inoculants to improve fertility in forage stands. Forage Technical Updates 2006. June 12, 2006, Saskatoon, SK (18 ppl)

J.D. Knight. Use of inoculants to improve fertility in forage stands. Forage Technical Updates 2006. June 12, 2006, Tisdale, SK (25 ppl)

K. Farden, and J.D. Knight. 2005. Strategies for Improving Soil Fertility in Mature Alfalfa Stands. Proceedings of the 2005 Soils and Crops Workshop. February 17-18, Saskatoon, Saskatchewan. On CD.

Personnel:

Kelly Farden, M.Sc. student, full time 2.5 years

Holly Bogard, summer student, 4 months, 2006

Wayne Kooy, summer student, 4 months, 2005

Janet Foley, technician, 2 months, 2005

Greg Tollefson, summer student, ½ time for 4 month, 2004

Darin Richman, technician, ¼ time, 2004

Equipment:

Digestion blocks for acid digestion of plant tissues and soils were refurbished; glass digestion tubes for the same digestions were purchased. This equipment was integral to this study.

Photographs:

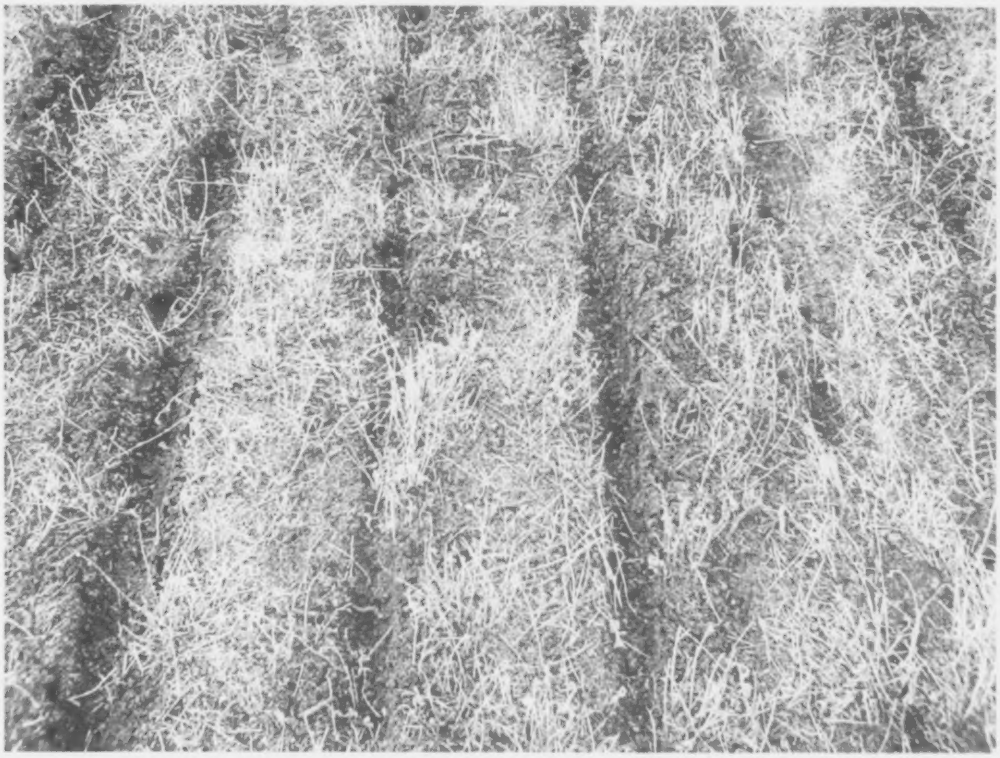


Plate 1. Soil disturbance (Crooked River site) caused by the disc seeder used to apply the P fertilizer and inoculant treatments to the mature alfalfa stands. Fertilizer and microbial treatments were applied as early in the spring as possible.

Plate 2. April 2005. Photograph of flooding at Aberdeen site. The site did not recover from the flooding.

Plate 3. Small plot seeder used for in-crop inoculation.

Plate 4. Star City site in July 2005 exhibiting severe sulfur deficiency symptoms. Plot on left is plot fertilized with ammonium sulphate, plot on right is unfertilized control.